BOTANY

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Volume LXXXI Number 4

The BOTANICAL GAZETTE

FOUNDED BY JOHN M. COULTER

JUNE 1926

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CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, London
THE MARUZEN-KABUSHIKI-KAISHA, Tokyo, Osaka, Kyoto, Fukuoka, Sendai
THE COMMERCIAL PRESS, LIMITED, Shanghai

Volume LXXXI Number 4

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of members of the botanical staff of the University of Chicago

Issued June 22, 1926

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, El Salvador, Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, and Spain. ¶Postage is charged extra as follows: For Canada and Newfoundland, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

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Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

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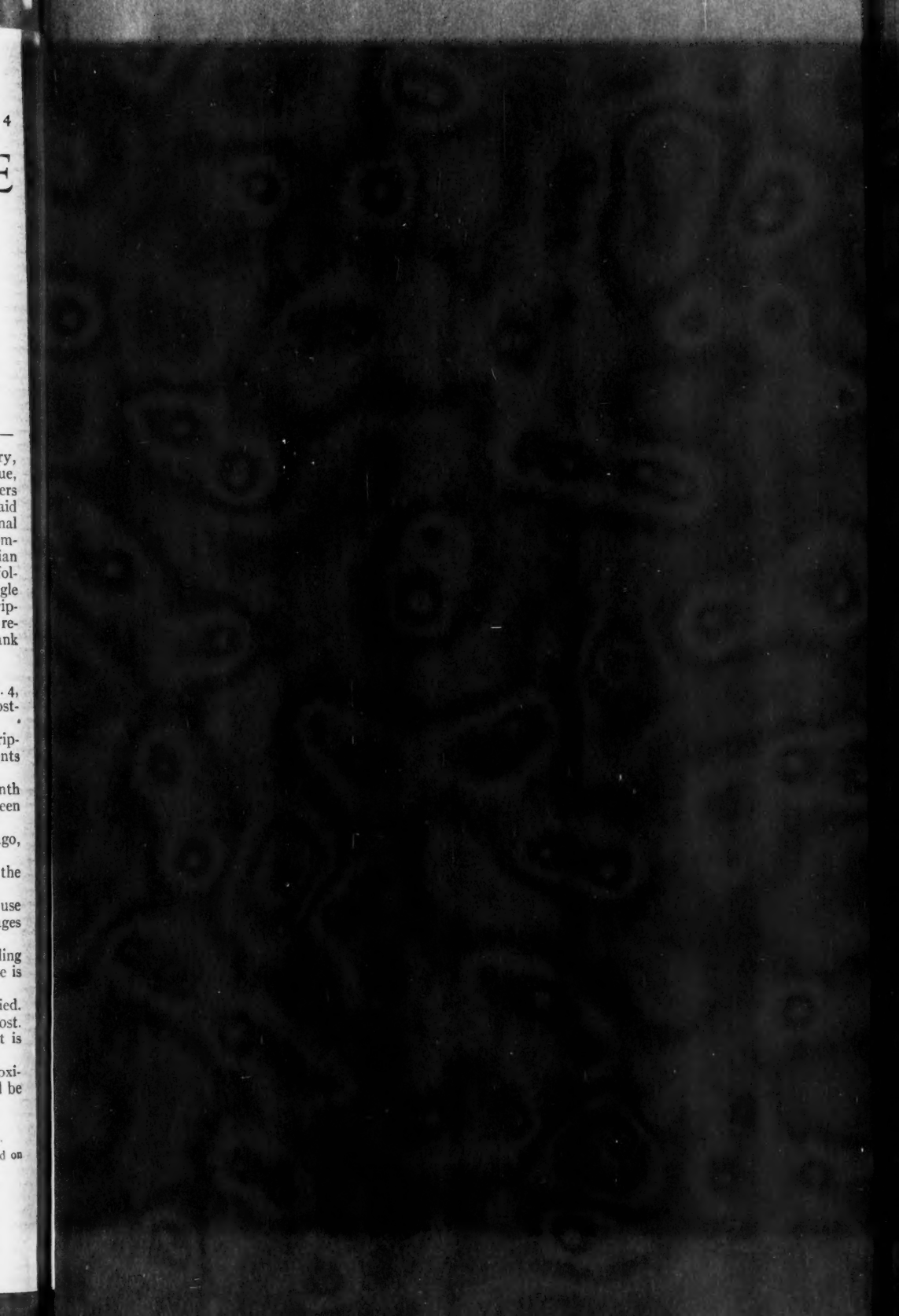
Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

PRINTED IN THE U.S.A



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For May 1926 and June 1926

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be a "second redaction."

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First
skrit

Arabic, by Abdallah Ibn Almokaffa (8th century), entitled *Calilah va Dimina* (names of two interlocutors), ed. Silvestre de Sacy, *Calila et Dimnah ou Fables de Bidpai* (1816).

Heb
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met

Persian, by Abou'l Maali Nasr Allah (12th century).

Spanish (Lost MS) used by Raimond of Béziers in his Latin version (*ca.* 1300 A.D.).

Greek by Sim Seth (*ca.* 1081), V. Puntoni (18

Lat
Dir

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Ital

Eng
pri

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Syriac version, whether made from Arabic or Pers uncertain, ed. W. Wright (1884).

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ca. 1081),
ntoni (18

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VOLUME LXXXI NUMBER 4

THE

BOTANICAL GAZETTE

June 1926

RELATION OF HYDROGEN-ION CONCENTRATION TO GROWTH OF CHLORELLA AND TO THE AVAILABILITY OF IRON[1]

E. F. HOPKINS AND F. B. WANN

(WITH FIVE FIGURES)

Introduction

In recent years there has accumulated considerable experimental evidence indicating that the growth of the higher green plants, as well as of fungi and bacteria, is influenced by the H-ion concentration of the culture medium. This has been demonstrated particularly in the case of some of the crop plants, where, of course, the results may prove to be of much practical importance. Field and pot experiments with higher plants, however, are always complicated by the presence of microorganisms in the soil, and by the difficulty of maintaining a constant H-ion concentration in the medium. Even when pure culture technique is employed, the complex structure of the plants used as experimental material renders a correct interpretation of the results difficult. Moreover, in many cases there is considerable uncertainty as to whether the effect produced by varying the reaction of the medium is one of H-ion concentration per se, or of the unavailability, as the result of precipitation, of some essential mineral element or elements of the nutrient solution. It occurred to the writers that the use of a unicellular green alga, grown in a nutrient solution of high buffer content, might simplify some of these

[1] This work was done at Cornell University under fellowships in the biological sciences, National Research Council. The writers wish to express their appreciation to both these institutions for the facilities which made the investigation possible.

difficulties and make possible a more exact analysis of the effect of the H-ion concentration on the rate of growth of a green plant.

The organism used in these experiments was a large celled species of *Chlorella*, of uncertain identity. This alga was obtained originally from soil, was isolated in pure culture, that is, one free from all other organisms, and for a number of years has been cultured in this laboratory on agar slopes.

Methods

The procedure adopted was to introduce uniform amounts of the pure culture of *Chlorella* into liquid culture solutions in Pyrex Erlenmeyer flasks of 150 cc. capacity. For each culture, 50 cc. of a mineral nutrient solution was supplied. Unless otherwise stated, the salt content of the solutions was the same in all cultures except for the varying amounts of the phosphate buffers used to secure the range of P_H desired. The dry weight of the crop produced after a definite time interval was used as a criterion of growth. It is realized that for certain types of solutions, the dry weights of the crops do not furnish a fair basis for growth comparison in *Chlorella*, due to the unequal starch content of the cells. Microscopic examination of the individual cultures of these experiments, however, did not show noticeable differences in this respect.

Experiment 1: Growth in complete nutrient solution at varying H-ion concentrations

The purpose of the first experiment was to determine the possible acid and alkaline limits for growth, as well as H-ion concentrations favorable for growth. For the cultures of this series a nutrient solution, designated as solution *A*, was prepared as follows:

$Ca(NO_3)_2 \cdot 4H_2O$	2.95 gm.
$MgSO_4 \cdot 7H_2O$	0.4 gm.
$Fe_2(SO_4)_3$	Trace
Glucose	20 gm.
Distilled water to	1000 cc.

Phosphate buffer mixtures, designated as solution *B*, were prepared from the following phosphate solutions:

M/7.5 KH_2PO_4	18.156 gm. per liter
M/7.5 K_2HPO_4	23.23 gm. per liter
M/7.5 H_3PO_4	13.07 gm. per liter

Various mixtures of these phosphate solutions were prepared, designed to give a range of H-ion concentration from P_H 2.8 to 7.13. Twenty-five cc. of solution *A* was introduced into each of a number of Erlenmeyer flasks and sterilized. To obtain the complete culture solutions of the desired H-ion concentrations, each of these portions of solution *A* was combined with a similar amount of sterilized solution *B*. This combination was effected with the usual aseptic precautions after both solutions were cool. The separate sterilization of solutions *A* and *B* prevented the caramelization of the sugar in the

TABLE I

COMPOSITION AND INITIAL P_H OF CULTURE SOLUTIONS FOR EXPERIMENT 1

No.	SOLUTION *A* (cc.)	M/7.5 H_2PO_4 (cc.)	M/7.5 KH_2PO_4 (cc.)	M/7.5 K_2HPO_4 (cc.)	INITIAL P_H ELECTROMETRIC	INITIAL P_H COLORIMETRIC
1	25	3.75	21.250	.....	2.81	.....
2	25	2.50	22.500	.....	3.07	3.30
3	25	2.00	23.000	.....	3.20	3.40
4	25	1.00	24.000	.....	3.47	3.60
5	25	.....	25.000	.....	4.14	4.30
6	25	.....	24.375	0.625	4.62	4.70
7	25	.....	23.750	1.250	4.94	4.95
8	25	.....	22.500	2.500	4.82(?)	5.05
9	25	.....	17.500	7.500	5.73	5.80
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11	25	.....	5.000	20.000	6.68	6.60
12	25	.....	1.250	23.750	6.95	6.90
13	25	.....	0.250	24.750	7.10	7.00
14	25	.....	.....	25.000	7.13	7.00

alkaline cultures, and to some extent the precipitation of magnesium and calcium phosphates. It will be noted that in the completed culture solutions the concentrations of salts in solutions *A* and *B* were decreased one-half.

Four replications of each H-ion concentration were prepared; one was used for the initial P_H determinations, and the remaining three in each case were inoculated. The H-ion determinations were made electrometrically and checked, except in the case of the most acid solution, colorimetrically, using GILLESPIE'S (4) drop ratio method. A bubbling type of electrode was found satisfactory in making the electrometric determinations. The apparatus was checked frequently, using an M/20 potassium hydrogen phthalate buffer mixture, as recommended by CLARK (1). The composition of the complete cul-

ture solutions and the data for the initial H-ion concentrations are given in table I. The initial P_H is also presented graphically in fig. 1.

INOCULATION.—The organism, *Chlorella* sp., was cultured on agar slants, and from these a suspension was prepared by scraping the cells from the surface of each slant and adding them to a sterilized balanced solution. This solution, which was used to prevent

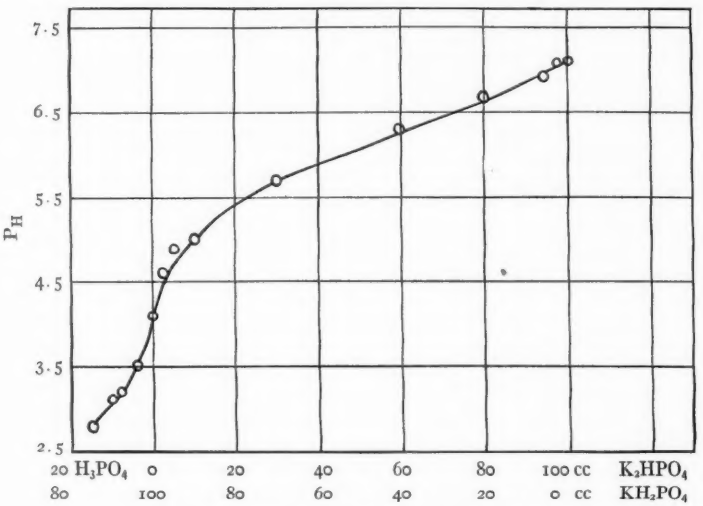

FIG. 1.—Composition-P_H curve of culture solutions used in Experiment 1: values of abscissae represent cc. of each constituent of buffer mixtures per 200 cc. of culture solution.

injury to the cells, was made up according to the method of OSTERHOUT (6), by mixing 95 cc. of a 0.6 per cent NaCl solution and 5 cc. of a 2.2 per cent solution of $CaCl_2$. To each culture flask to be inoculated, 0.5 cc. of this suspension was added, observing the usual aseptic precautions. The suspension was thoroughly agitated before taking sample. A microscopical examination of the algal cells in the balanced solution was made on the day following the inoculations, and they appeared to be normal.

The solutions were inoculated on April 15, 1924, and were placed at once on a shelf at the north window, where they were all quite

evenly lighted but never in direct sunlight. The first growth observations were noted down on April 24, nine days after inoculation. At this time considerable growth was observed in those solutions with

TABLE II

OBSERVATIONS NINE DAYS AFTER INOCULATING IN SOLUTIONS OF VARYING H-ION CONCENTRATION

CULTURE NO.	INITIAL P_H	NOTES
1.....	2.81	No growth; slight brownish precipitate
2.....	3.07	No growth; slight brownish precipitate, more than in no. 1
3.....	3.20	No growth; slight brownish precipitation, more than in no. 2
4.....	3.47	Growth (?); agglutinated precipitate
5.....	4.14	Growth, but slight and not very green
6.....	4.62	Good growth; distinctly green but with a yellowish cast
7.....	4.94	Good growth; more than in no. 6; healthier and more vigorous; slightly yellowish
8.....	5.05	Good growth; more than in no. 7; not as bright green as in no. 9
9.....	5.73	Probably the best growth; one culture contaminated with a fungus; bright green
10.....	6.31	Growth less than in no. 9; about like no. 8
11.....	6.68	No growth
12.....	6.95	No growth
13.....	7.10	No growth
14.....	7.13	No growth

TABLE III

EFFECT OF VARYING H-ION CONCENTRATION; EXPERIMENT 1

NO.	P_H AT START	P_H AT END			DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
		A	B	C	A	B	C	
1.....	2.81	2.84	2.84	.....	−0.9	−0.9	.....	0.0
2.....	3.07	3.04	3.04	.....	−0.2	+0.4	.....	0.1
3.....	3.20	3.11	3.12	3.14	+0.8	+0.8	−0.8	0.4
4.....	3.47	3.41	3.41	3.41	−0.4	−0.5	−1.2	0.0
5.....	4.14	4.61	4.48	4.35	19.9	13.3	9.0	14.1
6.....	4.62	4.83	4.77	4.83	65.4	52.6	37.3	51.8
7.....	4.94	5.05	5.04	4.99	93.4	84.4	62.4	80.1
8.....	5.05	5.10	5.10	5.10	105.8	116.9	67.8	96.8
9.....	5.73	6.69*	5.78	5.90*	177.9*	190.7	175.7*	190.7
10.....	6.31	6.30	6.37	6.34	146.1	110.6	149.5	135.4
11.....	6.68	6.66	6.50	.....	006.2	005.9	.....	006.0

* Contaminated.

an initial H-ion concentration in the vicinity of P_H 5.0, while in the very acid and somewhat alkaline cultures no growth was detected. The details of these observations are recorded in table II.

On April 29, fourteen days after inoculation, determinations were made of the dry weight of the crop produced in each culture, and of the final H-ion concentration of each solution. Each individual culture was added to a large Pyrex test-tube and the algal cells thrown down in the centrifuge. A portion of the clear supernatant liquid was poured off for P_H determination, and the residue made slightly acid in order to dissolve any precipitated salts. The algal cells were then

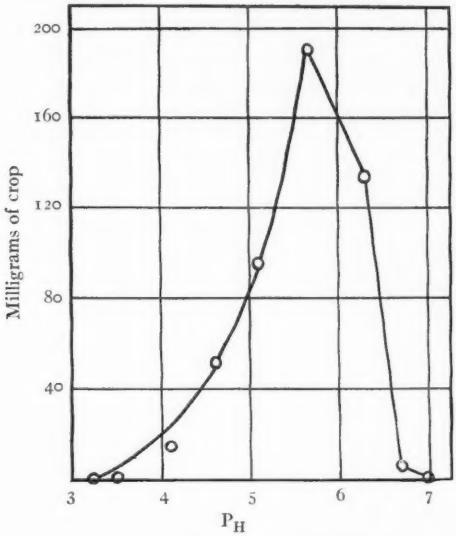

FIG. 2.—Growth-P_H curve of Experiment 1 with normal culture solution

thrown on a weighed Gooch crucible provided with a thoroughly washed asbestos mat, and the weight of the crop determined after drying *in vacuo* at 80° C. for 18 hours. The results are given in table III. In cases where contaminations occurred, the dry weights are not included in the averages. The crops were not determined in culture solutions nos. 12, 13, and 14, as no growth was apparent in any of the flasks.

It should be noted that the H-ion concentrations of the solutions remained very constant, even when there was considerable growth of the alga. In no case, except where contamination was a factor,

did the final P_H determination vary from the initial by more than 0.5, and in only seven cases was the variation greater than 0.1.

The data for the crop determinations are plotted in fig. 2, and show, so far as this experiment is concerned, that maximum growth occurred at P_H 5.7. The acid limit may be placed at about 3.4, and the alkaline at about 6.7. The curve representing the growth from 3.4 to 5.7 is very uniform.

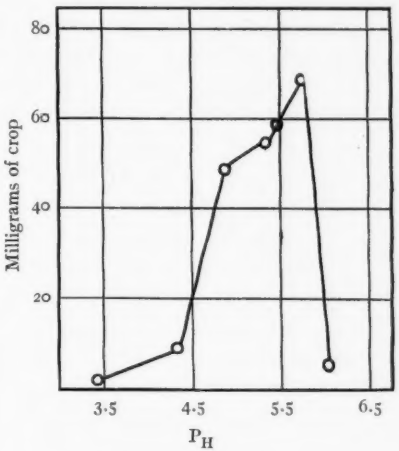

FIG. 3.—Growth-P_H curve of Experiment 2 with normal culture solution.

Experiment 2

In the experiment just described, the H-ion concentration values in the region of maximum crop production were rather widely separated, as an examination of the composition-P_H curve, fig. 1, will readily show. In an effort to obtain data on the growth of *Chlorella* over a closer P_H range in this region, and to check the results already secured, the experiment was repeated, using the same solutions and methods of procedure, but introducing a few more points in the P_H range. Culture solutions nos. 1, 2, and 3 on the acid end of the series, and nos. 13 and 14 on the alkaline end were omitted in this test because of the entire absence of growth in them. Four replicates of each culture solution were prepared as before, one flask of each lot being used for the initial H-ion concentration determination, and the remaining three being inoculated. The P_H determinations were made by means of the colorimetric method and checked in a few instances with electrometric determinations.

The inoculations were made on October 29, using 0.5 cc. of a suspension of *Chlorella* cells for each flask. Growth occurred in this series over practically the same range of P_H as in the previous ex-

periment, the best growth being at 5.7. At the end of three weeks the usual crop determinations were made. H-ion concentration determinations at this time showed very little change in the solutions. The data for the second experiment are presented in table IV, and are also shown graphically in fig. 3. The results confirm the point of maximum growth of *Chlorella* in this nutrient solution at P_H 5.7.

TABLE IV

DATA FOR EXPERIMENT 2: EFFECT OF P_H ON GROWTH

SOLUTION NO.	COMPOSITION: 25CC. SOLUTION A PLUS			INITIAL P_H	FINAL P_H			AVERAGE CROP (MG.)
	M/7.5 H_3PO_4 (cc.)	M/7.5 KH_2PO_4 (cc.)	M/7.5 K_2HPO_4 (cc.)		A	B	C	
4	1	24.000	.....	3.4	3.2	3.3	3.2	01.5
5	.....	25.000	.....	4.2	4.3	4.3	4.1	08.8
6	.....	24.375	0.625	4.9	5.3	5.3	5.2	48.1
7	.....	23.750	1.250	5.3	5.3	5.4	5.3	54.4
8	.....	22.500	2.500	5.5	5.5	5.5	5.5	59.3
8a	.....	21.250	3.750	5.7	5.7	5.7	5.9	68.5
9	.....	17.500	7.5	6.0	6.2	6.2	6.2	05.6
9a	.....	13.750	11.25	6.3	.....	.....	.....	.....
10	.....	10.000	15.0	6.6	.....	.....	.....	.....
10a	.....	7.500	17.5	6.7	.....	.....	.....	.....
11	.....	5.000	20.0	6.8	.....	.....	.....	.....
12	.....	1.250	23.75	7.2	.....	.....	.....	.....

PRECIPITATION OF SALTS OF SOLUTIONS

In both of the experiments, all the solutions on the acid side of P_H 5.7 were perfectly clear, whereas considerable precipitation occurred on the other side of this point. The precipitate consisted chiefly of magnesium and calcium phosphates, and it seems probable that the iron, supplied as ferric sulphate, was also largely thrown down. In the second experiment the amount of precipitation was greater than in the solutions of the first experiment, due no doubt to the lower temperature obtaining in the fall of the year. This fact probably accounts for the sharp drop in fig. 3, from 5.7 to 6.0. Because of this marked precipitation, the question arose as to whether lack of growth in these solutions was purely an effect of P_H, or might be due to the unavailability of some essential mineral elements. If the H-ion concentration alone were the factor involved, then

Chlorella should grow in these solutions containing heavy precipitates, provided the reaction was adjusted to the vicinity of P_H 5.5. On the other hand, magnesium, calcium, or iron may have been completely precipitated, resulting in solutions unfavorable for growth. Qualitative tests showed a mere trace of each in the filtered solutions. From the unpublished results of SCHRAMM,[2] it does not seem likely that lack of calcium would be a factor, as he has shown that good growth can be maintained in solutions from which calcium has been entirely omitted. Iron and magnesium, however, cannot be so completely eliminated without producing an effect. The following experiment was therefore devised to throw some light on this question.

Experiment 3: Growth in readjusted solutions

A liter of the complete nutrient solution was prepared in the usual manner, using a phosphate buffer mixture which gave an initial H-ion concentration of P_H 6.8. A copious precipitate formed, and the solution was allowed to stand until this had settled out completely. The precipitate was then filtered off and the clear filtrate adjusted to P_H 5.5, with concentrated HCl of low iron content (0.0001 per cent), 2.5 cc. of the acid being required. Fifty cc. of adjusted solution was introduced into each of sixteen flasks. These were divided into four lots of four flasks each, and treated as follows: (1) no additions; (2) one drop of $Fe_2(SO_4)_3$ solution to each flask; (3) 2 cc. 0.4 per cent $MgSO_4$ solution added to each flask; (4) 2 cc. 0.2 per cent $CaCl_2$ added to each flask. The flasks were then sterilized and inoculated with a suspension of *Chlorella* cells. The P_H after sterilization was 5.3.

As a check for this series, a number of flasks containing the unadjusted solution of P_H 6.8 were prepared in the usual manner. Some of these were inoculated at once; the contents of others were filtered through sterile filters into sterile flasks and then inoculated. After twelve days' growth, observations were recorded as follows: (1) fair growth, cells green at first but turning brown; (2) very good growth, bright green, healthy appearance; (3) good growth, bright green at first, turning to yellow-green; (4) slight growth, green at first, becoming practically colorless; (checks) no growth in unad-

[2] Paper presented before the Botanical Society of America, Boston Meeting, 1922.

justed solutions with heavy precipitate present; slight trace in unadjusted solution, filtered sterile. At this time P_H determinations showed that practically no change had occurred in the reaction of the solutions. Crop determinations were made, with the results shown in table V.

It is apparent that the unadjusted solution at 6.8 will not support growth. The small amount of growth in the filtered check may have been due to iron dissolved from the filter paper. The same solution, however, with reaction adjusted to P_H 5.3 permits of considerable development of *Chlorella*. In the light of later experiments

TABLE V

GROWTH IN READJUSTED SOLUTIONS

SOLUTION	TREATMENT	INITIAL P_H	FINAL P_H	AVERAGE CROP (MG.)
Unadjusted solution.....	None	6.8	.....	None
Unadjusted solution.....	Filtered	6.7	.....	01.8
Filtered and adjusted (1)...	None	5.3	5.1	05.9
Filtered and adjusted (2)...	+Fe	5.3	5.3	14.5
Filtered and adjusted (3)...	+Mg	5.3	5.3	13.0
Filtered and adjusted (4)...	+Ca	5.3	5.1	04.8

reported in this paper, it seems probable that this increase in growth is not due merely to the change in reaction, but to slight traces of iron added in the acid used in adjusting the solution after filtering. The addition of a small amount of iron or magnesium to the adjusted solution increased growth two or threefold, while the addition of calcium apparently depressed the growth. In solutions of P_H 6.0 and above, therefore, the precipitation of iron is a factor in the growth of the organism. Magnesium also may be involved. A trace of iron may have contaminated the magnesium solution used in adding this element, although it was not apparent from a qualitative test. Lack of calcium apparently is not a factor in prohibiting growth, while the depressing effect of its presence may be due to toxicity.

Experiment 4: Growth in Beijerinck's nutrient solution

One of the mineral nutrient solutions frequently used for the culture of green algae is that of Beijerinck, the composition of which, as modified by Moore, is as follows:

NH_4NO_3	0.5 gm.
K_2HPO_4	0.2 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
$CaCl_2$	0.1 gm.
$Fe_2(SO_4)_3$	Trace
Distilled H_2O	1000 cc.

This solution, either with or without the addition of agar or glucose, has proved very satisfactory for pure culture work with these forms. The reaction of the solution, however, is practically neutral, which would seem, from the results obtained above, to be unfavorable for growth. It seemed desirable, therefore, to examine the growth of *Chlorella* in this solution at various H-ion concentrations. The following experiment was thus devised.

TABLE VI

GROWTH IN ADJUSTED BEIJERINCK'S SOLUTION

SOLUTION	INITIAL P_H	FINAL P_H	AVERAGE CROP (MG.)
No sugar	6.9	6.4	2.9
	6.6	6.4	2.2
	6.5	6.2	2.7
	6.3	5.8	2.5
	5.3	4.0	2.1
1 per cent glucose	6.2	Less than 3.1	157.3
	5.8	Less than 3.1	137.0
	5.1	Less than 3.1	118.6
	4.1	Less than 3.1	116.2

A quantity of Beijerinck's solution was prepared, to half of which 1 per cent glucose was added. The P_H of the two portions, with and without glucose, was 6.9 and 7.0 respectively. The two solutions were then introduced individually into a number of Erlenmeyer flasks, in quantities of 50 cc. in every case, and the flasks sterilized. The solutions without glucose showed practically no change in P_H after sterilization; the solution with glucose had a P_H of 6.2 after sterilization. Several lots of the two solutions were adjusted in varying degrees toward the acid end of the P_H range by the addition of sterile HCl, and the whole series then inoculated with a suspension of *Chlorella* cells. At the end of a month determinations were made of the H-ion concentration of the solutions and of the dry weight of crops produced. The data are shown in table VI.

It is apparent that, although Beijerinck's solution may be nearly neutral when inoculated, it develops an acid reaction as growth proceeds, due probably to an unequal absorption of ions from the solution. Nitrogen is here supplied as NH_4NO_3, and it is conceivable that the NH_4 ion may be absorbed in excess of the NO_3 ion, thus producing the change in P_H. It should also be noted that there is not an adequate buffer system in this solution to maintain the reaction, as was provided in the experiments reported earlier in this paper. The fact that Beijerinck's solution will maintain a good vigorous development of green algae, therefore, is not evidence that a P_H of 6.8 or 7.0 is favorable for the growth of these forms.

Solubility of ferric iron in buffered culture solutions

With the idea in mind that the results obtained in previous experiments may have been caused by the insolubility of iron at the higher P_H values, some experiments were performed to determine the extent of this precipitation. At the same time an attempt was made to keep the iron in solution by means of salts of certain organic acids. The fact that lack of iron is a factor in alkaline solutions is indicated in Experiment 3, where the addition of iron to an alkaline culture solution after filtering and adjusting to a P_H of 5.5 increased the growth markedly over the check to which no iron had been added.

Phosphate solutions identical with those used in the previous experiments were mixed so as to give, when diluted with an equal amount of water, a buffer solution having a P_H of 7.0. At the same time 1 gm. of ferric sulphate was dissolved in 100 cc. of water, to which had been added three drops of concentrated hydrochloric acid. Various mixtures were then made, as follows:

Buffer solution (cc.)	Iron solution (cc.)	Water (cc.)	Other additions
25.....	1	25	None
25.....	1	25	0.5 gm. Na citrate
25.....	1	25	0.5 gm. K tartrate
25.....	1	25	0.5 gm. NaK tartrate

The last three mixtures were prepared in duplicate. At the time of mixing, all the solutions were turbid except the ones to which sodium citrate had been added. The next morning all had a small amount of flocculent precipitate with a clear supernatant liquid ex-

cept the ones with sodium citrate. In these no precipitate could be detected. These latter solutions were distinctly yellowish in color.

At this time the solutions were filtered through filter papers which had been washed thoroughly with distilled water, and the filtrates tested for iron by the procedure of MARRIOTT and WOLF (5).

The writers would like to point out that the extreme sensitiveness of this test for ferric iron makes it of great value in a study of the physiological effect of iron on plant growth, as amounts of iron well below that necessary for growth may accurately be determined. MARRIOTT and WOLF found that as little as 0.0005 mg. of ferric iron could readily be detected, and that differences such as between 0.002 and 0.003 mg. are easily determined. In our own experiments we also approached this same degree of accuracy. For instance, 0.00025 mg. of Fe could easily be distinguished from the blank and also from 0.0005 mg. It is imperative of course that the reagents used be free from iron, or at least have such a low iron content that a blank prepared from them have the very faintest trace of color when compared with a tube of water. The method slightly modified by us is as follows.

One cc. of concentrated hydrochloric acid of low iron content (0.0001 per cent) was placed in a 50 cc. graduated cylinder, and 10 cc. of a 10 per cent solution of KSCN, and 10 cc. or less of the solution to be tested added. The volume was then made up to 25 cc. with distilled water, and then to 50 cc. with acetone, and mixed. Comparison was made directly in this cylinder with a series of standards prepared in a similar manner in other 50 cc. graduated cylinders, and containing known amounts of iron. It was found convenient to place the cylinders on a glass plate supported above white paper, and compare the colors by looking down through the solutions. The concentration of hydrochloric acid used prevents the interference of phosphates (5). The standards were prepared from a stock solution made in the usual manner by dissolving pure iron wire.

The results of the preliminary tests on the filtered buffer solutions are shown in table VII. In the case of the check, the precipitate on the filter was dissolved in a little HCl and diluted to 50 cc., and also tested for iron. From this table it is seen that, after standing

over night, only about 1/100 of the iron is left in the phosphate buffer solution to which no organic salt was added. Practically all the iron is in the precipitate which forms. In the solution to which sodium citrate was added all of the iron remained in solution. The two tartrate salts used do not appear to be effective in holding the iron in solution.

TABLE VII

SOLUBILITY OF IRON IN PHOSPHATE BUFFER SOLUTIONS AT P_H 7.0

SOLUTION	FE IN 50 CC. (MG.)	P_H	
		Brom cresol purple	Phenol red
Check (filtrate)	0.028	6.9	6.9
Check (precipitate dissolved in HCl and diluted to 50 cc.)	2.800	.....	.....
Solution +0.5 gm. Na citrate (filtrate)	2.800	7.0	7.1
Solution +0.5 gm. K tartrate (filtrate)	0.056	6.9	6.9
Solution +0.5 gm. NaK tartrate (filtrate)	0.028	6.9	6.9

TABLE VIII

EFFECT OF SODIUM CITRATE ON SOLUBILITY OF IRON; OBSERVATIONS AFTER ABOUT 20 HOURS

SOLUTION	APPEARANCE	FE IN 50 CC. (MG.)	P_H
Check	White flocculent precipitate	Less than 0.028	7.4
+0.2 gm. citrate	Clear	.....	.....
+0.2 gm. citrate +Mg	Clear	.....	.....
+0.1 gm. citrate	Clear	.....	.....
+0.1 gm. citrate +Mg	Clear	.....	.....
+0.01 gm. citrate	Clear	.....	.....
+0.01 gm. citrate +Mg	Clear	.....	.....
+0.005 gm. citrate	Clear	2.8	7.4
+0.005 gm. citrate +Mg	Very slight turbidity	2.8	7.4

In another test using varying amounts of sodium citrate at a P_H of 7.4, it was found that this salt in as low a concentration as 0.005 gm. per 50 cc. of solution would hold the 2.8 mg. of iron in solution also. In this experiment magnesium sulphate was added in the same concentration as used in the culture solutions, to see whether this affected the solubility of iron. The results are given in table VIII. It is evident that only a small amount of the sodium citrate is necessary to keep the iron in solution, and also that magnesium does not interfere with its action.

Experiment 5: Effect of sodium citrate on availability of iron in normal culture solution

On the basis of the preceding experiments, another series of cultures was set up, using the same procedures as followed in Experiments 1 and 2, except that sodium citrate was added. The concentration of sodium citrate used was 0.4 gm. per liter of solution *A*. The final concentration was thus 0.2 gm. per liter of the culture solution as finally prepared.

In place of ferric sulphate, a standard solution of ferric chloride, prepared from pure iron wire and containing 0.1 mg. Fe per cc. was used. This was added at the rate of 8 cc. per liter of solution *A*, which amounts to 0.02 mg. Fe per culture flask containing 50 cc. of the final culture solution. As the actual tests of the solutions in Experiments 5 and 6 showed 0.0575 and 0.050 mg. Fe respectively per culture, it was thought that some iron must have been present as an impurity in the other reagents. From subsequent tests made on culture solutions with and without glucose, it appears that most of the additional iron was derived from impurities present in this sugar.

Six replications of the culture solutions were prepared for each P_H. Five of these sets were inoculated with *Chlorella* cells, and the remaining set used for initial H-ion determinations and iron tests as shown in table IX. On making the iron tests, it was found that, in spite of the presence of sodium citrate, the iron had unfortunately disappeared out of the more alkaline solutions. After standing over night no iron was found in the filtrates from cultures 8–14, and only small amounts in nos. 6 and 7. The others showed strong tests which indicated that none of the iron had been lost. The loss of iron in the more alkaline solutions may be accounted for by the fact that in these solutions an amorphous precipitate of calcium phosphate forms, and on this precipitate the iron is adsorbed. Thus, although the iron is present in a soluble form, it is removed from solution. This phenomenon should be distinguished from that occurring in the check solutions in the experiments just described (tables VII and VIII), where chemical precipitation of the iron occurs at the higher P_H values.

Providing that the iron removed by adsorption in this manner is unavailable for the growth of *Chlorella*, it was to be expected that

this series of culture solutions would give results little different from those obtained in Experiments 1 and 2; such proved to be the case. In making the inoculations of cultures 6–14 in each series of this experiment, an attempt was made to overcome this difficulty by adding in the cell suspension an amount of iron equivalent to that originally present. While, as the final iron tests show, most of this iron was also removed from solution, it appears that the shape of the growth curve is affected. The decline in the curve on the alkaline

TABLE IX

EFFECT OF SODIUM CITRATE ON AVAILABILITY OF IRON IN NORMAL CULTURE SOLUTION

SERIES NO.	P_H AT START	P_H AT END					FE INITIAL	FE FINAL MG. PER CULTURE SOLUTION			AVERAGE CROP (MG.)
		A	B	C	D	E		A	B	C	
1	3.60	.....*	3.7	3.7	3.70	3.7	+	.....*	0.0575	.....	1.95±0.23†
2	4.10	5.30	5.2	5.2	5.30	5.3	.....	0.0175	.....	.....	46.54±0.62
3	4.70	5.40	5.4	5.4	5.5	5.5	.....	0.0175	.....	.....	72.96±1.55
4	4.90	5.55	5.55	5.6	5.55	5.6	.....	0.0125	.....	.....	87.38±1.24
5	5.4	5.40	5.4	5.4	5.4	5.4	.....	0.00675	.....	.....	84.14±1.30
6	5.6	5.50	5.5	5.5	5.5	5.5	+	0.0075	0.015	.....	95.40±1.60
7	5.9	5.75	5.75	5.75	5.75	5.75	+	0.0175	0.020	.....	90.86±1.30
8	6.1	6.20	6.2	6.2	.....*	6.2	.....*	0.00177	<0.00125	.....	83.52±2.59
9	6.4	6.4	6.4	6.4	.....*	6.4	.....*	<0.00125	<0.00125	.....	43.85±1.10
10	6.7	....*	6.7	6.6	6.7	6.6	.....	.....*	<0.00125	<0.00125	7.07±1.19
11	6.9	6.9	6.9	.....*	6.9	6.9	.....	<0.00125	<0.00125	.....	1.07±0.33
12	7.05	7.0	.....*	7.1	7.1	.....*	.....	<0.00125	.....	.....	0.83±0.27
13	7.30	7.15	7.1	7.15	.....*	.....	.....	0.0075	.....	0.0075	1.9 ±0.83
14	7.40	7.2	7.2	7.25	7.15	.....	.....*	0.0125	<0.00125	.....	3.1 ±1.16

* Culture contaminated.

† Probable error of the mean calculated by means of Bessel's formula.

side is not so steep as in Experiments 1 and 2. The data are given in table IX and shown graphically in fig. 4. In this experiment, as well as in the following one, the cultures, after inoculation, were placed in the greenhouse but protected from direct sunlight. The cultures of each series were so distributed as to overcome effects due to unequal lighting. Crop determinations were made two weeks after inoculating.

The greatest growth is at P_H 5.7 as before, and falls off very rapidly as the solutions become more alkaline. As in the case of the previous experiments, however, this cannot be looked upon as the true effects of H-ion concentration on growth because of the variation in the iron content of the nutrient solution. This is brought out

very strikingly by the data obtained in Experiment 6, in which we were successful in keeping the iron in solution in all cultures.

Experiment 6: Effect of sodium citrate on availability of iron in culture solution lacking calcium

As the work of SCHRAMM, previously referred to, shows that very little if any calcium is necessary for the growth of *Chlorella*, it was decided to set up a series in which the calcium nitrate of the nutrient

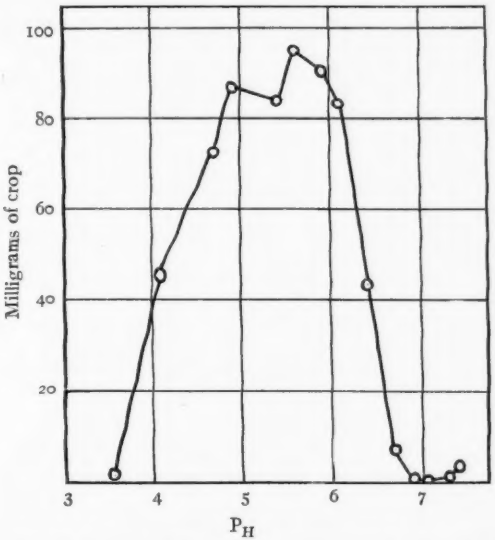

FIG. 4.—Growth-P_H curve of Experiment 5 with normal culture solution to which sodium citrate was added.

solution was replaced by ammonium nitrate containing an amount of nitrogen equivalent to that supplied by the calcium nitrate in the other series. It was found that in solutions prepared in this manner no precipitate forms at any of the H-ion concentrations used. Under these conditions, with sodium citrate present, the iron is neither precipitated chemically nor removed by adsorption. The growth should therefore be a function of the H-ion concentration.

Six replications of the culture solutions were prepared, and except

for the substitution mentioned, the same procedure was followed as in Experiment 5. In preparing the suspension of algal cells, instead of a balanced solution, a 0.6 per cent NaCl solution was used. Crop determinations were made two weeks after inoculating. The data for this experiment are summarized in table X. It will be noted that the initial iron tests show as much iron in the most alkaline solution

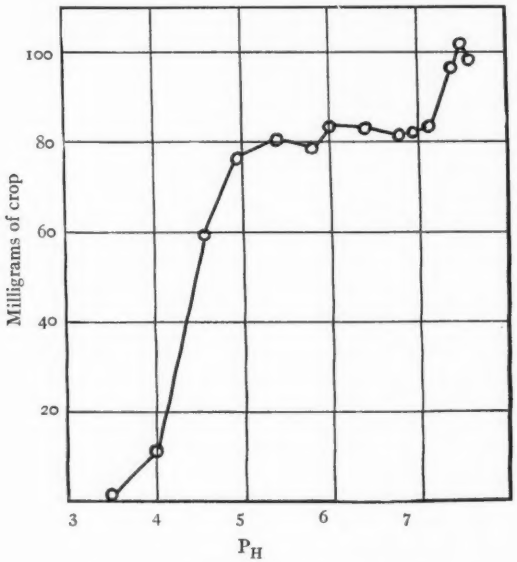

FIG. 5.—Growth-P_H curve of Experiment 6 in which calcium of normal culture solution was replaced by ammonium, and sodium citrate was added to keep the iron in solution.

(7.5) as in the most acid solution (3.5). The most interesting thing shown by these data, however, is the effect of the H-ion concentration on growth. Instead of a rapid falling off in the growth from P_H 5.7, it continues at approximately the same rate as the more alkaline solutions are reached, and even increases still more at the values of 7.4 and 7.5 (fig. 5).

The data for this experiment show that in most cases there is a slight increase in the acidity of the solution, probably due, as

postulated earlier, to a differential absorption of the ions of ammonium nitrate. In order to test this experimentally, analyses of the solutions were made at the end of the experiment for ammoniacal nitrogen and total nitrogen. The difference between these two results will give the amount of nitrate nitrogen. Ammoniacal nitrogen was determined by means of a modified Folin method, and the total nitrogen with Devarda's alloy.

TABLE X

EFFECT OF SODIUM CITRATE ON AVAILABILITY OF IRON IN CULTURES MINUS CALCIUM

SERIES NO.	P_H AT START	P_H AT END					FE INITIAL MG. PER CULTURE	FE FINAL MG. PER CULTURE A	AVERAGE CROP (MG.)
		A	B	C	D	E			
1	3.50	3.6	3.6	3.7	3.7	3.6	0.050	0.044	1.08±0.09
2	4.00	4.2	4.2	4.2	4.2	4.2	.....	0.048	11.06±1.68
3	4.65	4.4	4.4	4.45	4.3	4.4	.....	0.044	59.30±1.23
4	4.95	4.6	4.6	4.45	4.4	4.6	.....	0.044	76.52±1.15
5	5.4	4.8	4.7	4.7	4.65	4.7	.....	0.044	80.88±2.17
6	5.8	5.4	5.4	5.4	5.4	5.4	.....	0.0180	78.84±1.01
7	6.0	5.65	5.65	5.65	5.65	5.75	.....	0.0230	83.32±0.80
8	6.4	6.0	6.1	6.0	6.2	6.1	.....	0.0230	83.02±1.31
9	6.8	6.4	6.4	6.4	6.4	6.4	.....	0.0345	81.48±0.95
10	6.95	6.6	6.6	6.5	6.5	6.6	.....	0.0184	82.20±2.00
11	7.15	6.8	6.8	...*	6.8	6.8	.....	0.0253	83.40±3.18
12	7.4	6.9	6.9	6.9	...*	6.9	.....	0.0184	96.45±3.67
13	7.5	6.9	7.0	7.0	7.0	6.95	0.050	0.0253	101.96±4.32
14	7.6	7.0	7.0	7.0	7.0	7.0	0.050	0.0184	98.24±2.55

* Culture contaminated.

From table XI it appears that in all cases except nos. 1 and 2, in which cultures the reaction did not become more acid, there is a greater absorption of the ammonium ion than the nitrate ion. This would account for the reaction becoming more acid, and would also appear to justify our explanation of the increased acidity in the case of Beijerinck's solution. The varying amounts absorbed are due to differences in the amount of growth, and perhaps also to the differences in H-ion concentration. It is recognized, of course, that in alkaline solutions the liberation of gaseous ammonia may introduce an error in the determinations. As our most alkaline cultures were only slightly more alkaline than neutrality, however, it does not seem likely that this would occur.

In order to determine whether the lack of calcium in the culture solution caused any decrease in the crop, two series of cultures were prepared at the same P_H. One series contained calcium, and the composition was the same as in culture 5, Experiment 5; the other series

TABLE XI

DIFFERENTIAL ABSORPTION OF IONS OF AMMONIUM NITRATE FROM CULTURE SOLUTIONS

CULTURE NO.	AMMONIACAL NITROGEN (MG.)		NITRATE NITROGEN (MG.)	
	In solution	Absorbed	In solution	Absorbed
1 D	4.10	0.15	3.89	0.36
2 D	3.83	0.42	3.75	0.50
3 D	2.58	1.67	3.15	1.10
4 D	2.09	2.16	3.14	1.11
5 D	1.77	2.48	2.32	1.93
6 D	1.37	2.88	1.48	2.77
7 D	1.42	2.83	3.89	0.36
8 D	2.04	2.21	3.74	0.51
9 D	2.24	2.01	3.09	1.16
10 D	2.40	1.85	3.81	0.44
11 D	2.19	2.06	4.11	0.14
12 D	1.91	2.34	4.37	.....
13 D	1.78	2.47	4.38	.....
14 D	1.69	2.56	4.44	.....
Original solution	4.25	.....	4.25*	.....

*Calculated.

TABLE XII

EFFECT OF OMITTING CALCIUM FROM CULTURE SOLUTION AND REPLACING IT BY AMMONIUM

CULTURE NO.	CALCIUM PRESENT		NO CALCIUM	
	Crop (mg.)	P_H (final)	Crop (mg.)	P_H (final)
1	220.8	5.6	257.0	5.1
2	228.5	5.6	243.8	5.0
3	242.8	5.6	273.8	4.8
4	224.5	5.6	265.9	4.7
Average	229.1 ± 3.2	5.6	260.1 ± 4.3	4.9

contained no calcium, and the composition was the same as culture 5, Experiment 6. The initial P_H was 5.4 in both series, and no precipitate was present in any of the solutions. The cultures were inoculated March 17, 1925, and the crops and final P_H determinations

were made April 23 following. The results presented in table XII, instead of showing a decrease when calcium is omitted, show a marked increase over the series in which calcium is present. It is of interest to note that, while in the series in which calcium is present there is a slight increase in P_H, in the other series where calcium is replaced by ammonium there is a marked decrease. This is in keeping with the results obtained in Beijerinck's solution and in Experiments 5 and 6. This increase in acidity in cultures containing ammonium nitrate has already been discussed. The greater increase in the Beijerinck's solution is due to the fact that this is not so well buffered as in the other experiments.

Discussion

From a consideration of the experiments reported in this paper, it is obvious that if precautions are not taken to keep the iron of the culture medium in solution, the results obtained by varying the H-ion concentration will not represent the true effect of this factor on growth. Within a certain acid range, of course, no special efforts are needed to retain the iron in a soluble form; it is in the more alkaline solutions that it may be precipitated and its concentration so reduced as to be less than that required for growth. In Experiments 1 and 2, for instance, where the iron was precipitated chemically and probably also adsorbed to some extent, and in Experiment 5 where it was no doubt removed mostly by adsorption in the more alkaline cultures, there appeared to be a definite maximum for growth at about P_H 5.7, from which point the growth rapidly declined as the reaction became more alkaline, until at 7.0 there was no growth. In view of the results obtained in Experiment 6, however, where the iron was kept in solution at all P_H values, and also from the fact that most of the iron was found to be lost from the alkaline cultures of Experiments 1, 2, and 5, it is seen that except for reactions more acid than P_H 5.7, this does not represent a real effect of H-ion concentration on growth. In Experiment 6 growth continues to increase, even at 7.5. The alkaline limit for the growth of *Chlorella* has not been determined, therefore, and it remains for further work to decide this point.

The unavailability of iron in nutrient solutions at the higher P_H

values has been discussed by a number of workers, among whom might be mentioned TOTTINGHAM and RANKIN (8), and REED and HAAS (7). Earlier literature will be found cited in these papers. The general facts brought out are that iron is precipitated at the lower H-ion concentrations; that its precipitation is more rapid the lower the H-ion concentration; that the iron of certain iron salts is less likely to be precipitated than that of others; and that certain salts of organic acids tend to keep the iron in solution. Among the most favorable forms of iron appears to be ferric citrate, and it has been shown that this salt tends to remain in solution. DUGGAR (3) has shown that "soluble ferric phosphate" is an excellent source of iron, but as this substance is prepared by mixing ferric citrate and sodium phosphate (2), it is probably equivalent to adding ferric citrate to solutions in which the other ions are already present. In our experiments, where sodium citrate was added to the culture solutions, ferric citrate was probably present. It is possible that, if the iron in this case is present in colloidal solution in a condition of high dispersity, as suggested by DUGGAR, the citrate ion may act as a peptizing agent tending to maintain this condition. An excess of sodium citrate as used in our experiment may therefore be desirable. REED and HAAS show that where sodium citrate is added to a culture solution the iron is retained in solution, so that an "abundant" test is obtained with KSCN at P_H 7.6, while the control shows "none." While this result was qualitative, it shows that considerable iron at least was held in solution. Their test seems to differ from ours, in that they report a white turbidity where the sodium citrate was added, while ours was clear with a faint yellowish color.

An important point brought out in Experiment 5 is the effect on the iron concentration of an amorphous precipitate, calcium phosphate, even when the iron is present in a so-called soluble form. As this precipitate is frequently formed when nutrient solutions are made alkaline, it may account for the discordant results reported in the literature as to the availability of certain forms of iron.

As regards the solubility of iron in alkaline cultures where no sodium citrate was added, the high concentration of phosphates used to buffer the solutions may have decreased the solubility of iron, if this were present in the solutions as ferric phosphate, because of

their effect on the solubility product. We might therefore expect less iron in these solutions than in ordinary unbuffered culture solutions at the same P_H. The high concentration of phosphate does not appear to affect the solubility of iron when sodium citrate is present.

In investigating the availability of iron at varying concentrations of the H-ion, changes in the P_H value of the solution during the course of the experiment should be taken into account. This is especially true in unbuffered solutions. This is brought out by REED and HAAS in connection with the use of ammonium salts of organic acids which caused a marked increase in the H-ion concentration. Experiment 4, in which Beijerinck's solution was used, showed a marked increase in H-ion concentration during the growth of the organism, due, as subsequent tests showed, to the rapid absorption of the ammonium ion in preference to the nitrate ion of the ammonium nitrate. This increase in acidity will cause a marked increase in the solubility of iron. In strongly buffered solutions, such as used in Experiment 6, this effect is slight.

Summary

1. The rate of growth of *Chlorella* in highly buffered nutrient solutions is directly influenced by the H-ion concentration when the P_H is less than 5.7. The acid limit for growth of this organism in the culture media tested is 3.4.

2. In solutions in which the P_H is higher than 5.7, the availability of iron becomes a limiting factor for growth.

3. Certain organic compounds, especially sodium citrate, are effective in holding iron in solution in alkaline buffer mixtures of 7.4. When calcium is present, however, the iron is completely removed from such solutions by the precipitated calcium phosphate, probably as the result of adsorption.

4. Since calcium is not essential for the growth of *Chlorella* it can be omitted entirely from the culture solution. Iron can then be maintained in alkaline solutions in a form available for growth by the addition of sodium citrate. In such solutions maximum growth occurred at 7.5. The alkaline limit for growth has not as yet been established.

5. In unbuffered nutrient solutions, marked changes in the H-ion concentration may be brought about by the unequal absorption of ions, resulting in increased acidity, which in turn renders the iron available for growth.

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SPOROGENESIS IN REBOULIA HEMISPHAERICA

MARY CONSTANCE BLAIR

(WITH PLATES XXX, XXXI AND THREE FIGURES)

Historical review

The earliest contributions upon sporogenesis, by HOFMEISTER, LEITGEB, and LECLERC DU SABLON, were upon morphological characters wholly. FARMER'S (9) paper in 1893 marked the beginning of cytological investigation in the Bryophytes. *Aneura pinguis* and *Pallavicinia decipiens* were shown to reduce by a quadripolar spindle, which effected a simultaneous distribution of the chromatin to four separate points. In 1894 FARMER (10) published a substantiating investigation for *Pallavicinia*, but in *Aneura* he found that the quadripolar spindle was transient, and that it gave way to two simultaneous spindles that effected reduction by joint action.

FARMER'S (11) greatest contribution appeared in 1895, as a comparative study of several types, with confirmatory observations upon additional species. *Fossombronia Dumortieri* and *Pellia epiphylla* (anacrogynous Jungermanniales) were found to possess a prophasic quadripolar structure, which in metaphase did not become a quadripolar spindle. The evolved bipolar spindle arose by the fusion, in pairs, of the arms of this quadripolar structure; this was effected by the activity of four marked centrospheres or centrosomes with astral radiations. A complete septum followed this spindle. With no period of interkinetic rest, two simultaneous bipolar spindles completed reduction.

In the Marchantiales, *Fegatella conica* showed no lobing of the mother cell and no quadripolar figure. The early heterotypic spindle was triangular, having the block of chromosomes at one angle, and a very distinct centrosphere (with aster) at each of the other angles. By their progressive migration, the bipolar spindle was established with a straight axis. With no period of interkinetic rest, duplicate simultaneous spindles completed the reduction division. Cell plates preceded spore wall formation. Confirmatory examples were af-

forded in *Riella*, *Plagiochasma*, and *Marchantia*. The results secured in this investigation led FARMER to postulate a theory of phylogenetic advance in the reduction processes of plants. To him the Bryophytes furnished several transitional phases of marked significance.

MOORE (**25**) investigated *Pallavicinia Lyellii*, with very different results from those attained by FARMER in *P. decipiens*. The first spindle was bipolar, and had no cell plate. Eight tetrads arose from a split spireme. The kinoplasm arose as caps attended by marked nuclear distortion, and the nuclear membrane was resolved into spindle fibers. There was no interkinetic rest. No nuclear membrane appeared. The subsequent, simultaneous homotypic spindles established cell plates.

DAVIS (**7**) investigated *Anthoceros laevis* L. and discovered no centrospheres and no quadripolar spindle, but there was a true bipolar spindle. The daughter nuclei attained a fully developed resting condition. Homotypic spindles followed. The rather unusual findings included: (1) a spireme unchanged in character post-synaptically; (2) the laying down of the spore wall by a Hautschicht layer without the aid of spindle fibers or the formation of cell plates.

DAVIS (**8**) made a detailed investigation of *Pellia epiphylla*. He found the quadripolar spindle to be a transitory feature of prophase, and to be unattended by centrospheres. Granular protoplasm was the only form of kinoplasm permanent in the cell. Double longitudinal splitting of the chromatin was not found, and meiosis was effected by one heterotypic followed by two homotypic spindles. In other stages of ontogeny, centrospheres and polar caps were found.

BEER (**2**), working on *Riccia glauca*, found a continuous post-synaptic spireme, which was thickened at the expense of a nucleolus that was always distinct among indefinite linin fibers. The spindles were successive, and all were followed by membranes developed from cell plates.

LEWIS (**20**), investigating *Riccia lutescens* and *R. crystallina*, found neither kinetic bodies nor multipolarity. No nucleolus appeared, and the chromatin massed excentrically in synizesis. Nuclear elongation established the poles of the spindle. There was a cell plate, but not reorganization of nuclei, nor interkinetic rest.

In *Riccia Frostii*, BLACK (3) found scant chromatin and linin, but there was a very definite nucleolus. The spindle arose from extra-nuclear material, and it developed an incomplete cell plate. None followed the homotypic spindles. Centrospheres were not found.

VON MEYER (21) found only a smooth chromatin nucleolus in *Corsinia marchantioides*, which roughened as it fragmented. Linin and chromatin connected freely with the nuclear membrane. An incomplete suspended cell plate after the first spindle was complemented by the secondary cell plates of the homotypic spindles. Spore protoplasts were cut out by their centrifugal growth. Later VON MEYER (22) found no very radical departure from this in *Plagiochasma rupestre*. The chromosomes were thought to be derived from a fragmented nucleolus.

GRAHAM (15) investigated *Preissia commutata*. A diplotene, presynaptic, continuous, chromatin-bearing spireme by diakinesis resulted in eight split, bivalent chromosomes. The nucleolus disappeared, giving rise (perhaps) to spindle fibers. The heterotypic spindle arose from hyaline polar caps, and it was followed by a cell plate. Reconstruction but not rest preceded two simultaneous, homotypic spindles.

Papers confirming reduction by successive bipolar spindles include: GARBER 1904, on *Ricciocarpus natans;* JOHNSON 1904, on *Monocloea;* HUMPHREY 1906, on *Fossombronia longiseta;* CLAPP 1912, on *Aneura pinguis;* MCCORMICK 1915, on *Symphogyna aspera;* FLORIN 1918, on *Chiloscyphus polyanthus*. Confirming quadripolar spindles are: CAMPBELL 1913, on *Calycularia radiculosa;* also 1914 on *Pallavicinia radiculosa* and *P. Zollingeri;* GRÜN 1914, on *Treubia insignis*. Subsequent to the brief observations of LECLERC DU SABLON in 1885, the only previous work on sporogenesis in *Reboulia* was that done by HAUPT (18) in 1921. In that study were discussed the general morphological details of the spore mother cells, the elaters, the tetrads, and the spores.

JUNGERMANNIALES.—Briefly to summarize the situation, we find the feature of the lobing of the spore mother protoplast to be undisputed. Excentric and lobed nuclei were reported only by DAVIS (8) and FLORIN (14). With the exception of some minor details, syni-

zesis or the first contraction is granted universally. Whether a true synapsis of parental spiremes occurs at that period is still debatable; FARMER heads the negative side. It is a matter of common agreement that diakinesis cuts out bivalent chromosomes from a chromatic spireme. The first separation is a transverse or reducing division (DAVIS **8,** contra) in the sense common to sporogenesis in Spermatophytes. The second separation is a longitudinal or equational division. Whether there is complete, double, preparatory splitting of the chromosomes in prophase is a question that is rapidly being answered in the negative. The opposing school includes FLORIN and FARMER. The views of the latter are based upon the concept of a quadripolar spindle, typical for *Pallavicinia.* This structure is not given more than transitory value by more recent investigators. Most research indisputably reveals the presence of successive spindles in meiosis, but much variation is reported in the phenomena of interkinesis. It is evident that the terms "cell plate," "resting period," and "reorganization" need a more precise definition. The prevalent verdict is that rest, reorganization, and incomplete septa mark the period of interkinesis.

As regards kinoplasmic features, it is generally thought that a special zone comes to surround the nucleus in prophase. Its premetaphase manifestations are generally described as being of the independent multipolar type common to Pteridophytes and Spermatophytes. The only investigator to see specialized kinetic bodies in spore mother cells was FARMER (**11**). Centrospheres accompanied by centrosomes (*Pellia, Pallavicinia,* and *Fossombronia*) were found both here and in sporogenous and vegetative tissues. CHAMBERLAIN (**6**) and DAVIS (**8**), working with *Pellia*, found marked centrospheres in the germinating spore. Confirming this evidence for the presence of central bodies, FLORIN (**13**) has recently reported distinct centrosomes and asters in *Riccardia* (*Aneura*) after pairing of the pronuclei.

That all these bodies govern spindle formation and activity is granted. They are thought to arise in the cytoplasm *de novo*, and usually independently of each other. Together with the kinoplasm, they resolve into undifferentiated cytoplasm at the end of each mitosis. The chromosome count is becoming standardized at eight

for the gametophyte. The acrogynous Jungermanniales have still to be investigated.

MARCHANTIALES.—Barring the early amoeboid condition noted by HAUPT (18), the spore mother cells are spherical, *Fegatella* excepted. FARMER (11) and Miss MCCORMICK (23) reported contrary findings. Prophase phenomena do not feature the quadripolar figure. The spindles are bipolar, and originate from multipolar foci.

Instead of the four simultaneous centrospheres observed in the Jungermanniales, FARMER (11) here reported but two in *Fegatella*. No other worker has reported them in this phase of ontogeny. GRAHAM (16) has reported true centrosomes in fertilization for *Preissia* and also in stages of the young sporophyte up to four cells. In *Marchantia,* IKENO reported centrosomes as a constant feature of spermatogenous cells. MOTTIER (26) and VAN HOOK (28) found them in the gametophore of the same plant. The gametophytic chromosomes number eight generally. The further history of reduction differs in no appreciable way from that of the Jungermanniales.

Introduction

In undertaking the cytological problems of *Reboulia*, there would be no expectation of finding kinetic bodies in its ontogeny, with the possible exceptions of during the period of fertilization and in the germinating spore. The question of the quadripolar spindle would be eliminated, since all other Marchantiales display a reduction effected by two successive and independent mitoses.

As regards the early intent to prove a series of developments in the kinoplasmic architecture of the cell, phylogenetically significant from the lower Hepaticae up through the higher Hepaticae, as based upon comparative phases of ontogeny in all the genera, it must be said that such attempt has not succeeded signally. The accumulating evidence points to uniformity, and not to interpretations of cyto-phylogenetic difference. Much work will be required to arrive at any worthy conclusion in this matter; accordingly no discussion of this topic will be undertaken in this paper.

In the sporogenetic history, matters upon which there exists a wide variation of opinion include the origin of kinoplasm; its subsequent organization; the source and disposition of the chromatin;

the organization of the chromosomes; the source, character, and mutual relationships of nucleolus and chromatin-reticulum; the character and location of metaplasmic bodies; the precedence and extent of cell septa, and their part in cytokinesis and spore wall formation. To discuss these matters is the chief purpose of this paper.

Materials and methods

The material upon which this study of the sporogenetic processes in *Reboulia hemisphaerica* (L.)Raddi is based, was secured from Miner's Gully, Illinois, about 140 miles west of Chicago. This is a gulch of considerable extent, and is situated in the unglaciated portion of Jo Daviess County. Fixation in the field was not always feasible, so that some material had to be brought to maturity by means of outside bedding.

Fixation in the field made on April 9, 1921, yielded no studies in the reduction division. Transferred material (fixed daily) yielded the first spindles on April 19. This probably antedated reduction in the field by several days. In 1922 the late spring deferred reduction in outside bedded material to May 10. In favorable seasons *Reboulia* undoubtedly has completed its reduction divisions by the end of April.

Fixation in the field gave the best results, although transferred material did well if the specimens were kept in a turgid condition up to the time of immersion. Excision of the sporophytes proved to be difficult, and was found to be unnecessary. Incision of the dome of the carpocephalum secured rapid penetration. Any aperture made in the sporophyte resulted in the loss of the spore mother cells. A quick plunge into 95 per cent alcohol sufficed to dissolve any superficial protective substances.

Persistent efforts failed to devise any successful method for the use of the osmic series of fixing fluids. No bleach was of any service. In synizesis, MOTTIER'S mitochondrial formula gave admirable results when stained with safranin in conjunction with salkind blue. CHAMBERLAIN'S formula for the germinating spore of *Pellia* proved usable when the spores neared maturity. Outside of these two periods, no recommendation can be made for the employment of osmic acid as a fixing agent in the reduction division of *Reboulia*.

The best results were secured from the use of the chromic series of fixing agents. Stock solution gave the most delicate fixation. Bouin's fluid gave brilliant pictures, but resulted in considerable cell enlargement.

The most abundant metaphases were found at 11:00 A.M. The actual meiotic processes of any given sporophyte are accomplished within a very limited period, probably less than two days. To secure post-reduction phenomena required fixation for a much more prolonged period. The chloroform-paraffin method was used. Sections were cut 2–20 μ thick. Seven μ afforded the most serviceable material for the general study of cytological detail.

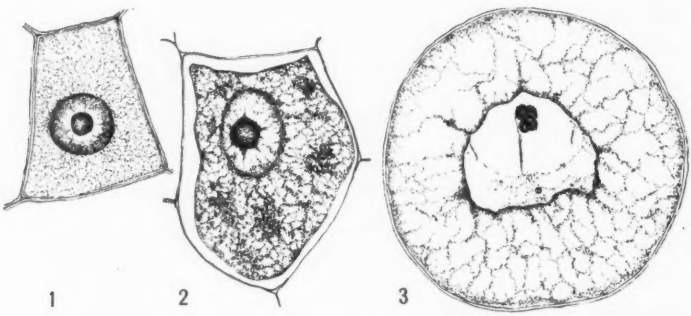
The inability to employ osmic fixation made the task of securing an adequate stain a difficult one. Iron-haematoxylin was not easy to employ because of the avidity with which the cytoplasm absorbed it. The chromatin also showed a tendency to accumulate the black pigment, and it was not retained well in the process of destaining, as the cytoplasm unfortunately held the haematoxylin much longer than did the chromatin. Mitochondrial stain proved most inapplicable. Flemming's triple stain gave satisfactory results, but the period in safranin had to be prolonged to two days; that in gentian violet to four hours. In sections thicker than 5 μ, where background was desirable, alcoholic light green proved to be a most effective substitute for Orange G.

Results

This study starts with the resting condition of the last sporogenous division. Figs. 1 and 2 represent early sporogenous cells from separate receptacles on a single thallus, the anterior one (fig. 1) bearing final sporogenous tissue, and the posterior (fig. 2) early meiotic tissue. Subject to the same growth environments, the same fixation, and the same stain on a single slide, they furnish authentic and comparative data. The sporogenous mass was a definite tissue, ellipsoid in transverse section, and measuring roughly 12 by 16 cells in its broadest diameters. Having used mitochondrial fixation (MOTTIER) and stain (WILSON), the tissues were so loaded with metaplasmic elements that finer details were obscured; but the vegetative tissues revealed that the action of the fixing fluid had been gentle. Fig. 1 shows the distinct walls of the sporogenous cells, and the dense

cytoplasm firmly inclosing the large spherical nucleus. The latter was turgid, and it contained a large, orbicular, deeply staining nucleolus. The linin, with extremely fine chromatin granules staining blue, appeared as a very tenuous film at the nuclear border, the nucleolus being centered within a broad hyaline areola. These nucleoli gave the tissue a sharply punctate appearance.

The honeycomb character of the tissue can be appreciated better from fig. 2. This represents a cell from the older and posterior sporophyte, in which considerable expansion had occurred without any



FIGS. 1–3.—Fig. 1, final sporogenous cell, resting condition; fig. 2, similar cell exhibiting meiotic prophase activities of growth and retraction from sporogenous cell wall; fig. 3, new spore mother cell wall; protoplast shows marked changes in size, form, and cytoplasmic arrangement; same magnification as figs. 1 and 2.

increase in the number of sporogenous cells. Near the center of the sporogonium the protoplasts had begun retracting from their sporogenous walls, which no longer stained as deeply nor appeared so thick as before. The continued stretching of these walls, induced by the progressive growth of the sporophyte, alters their angularity until the elongation of the elater cells ruptures the whole fabric. However, highly significant changes had occurred in the spore mother cell as the cytoplasm, replete with deeply staining metaplasm, had become vacuolated and coarsely stranded. This change indicated preparation on the part of the protoplast for the immediate elaboration of the future wall of the spore mother cell. This wall seemed to be created by an effusion of substance elaborated by the multitudinous granules of the peripheral portion of the proto-

plast. Capsular enlargement obtained progressively throughout the period of wall formation, but the mother wall thickened so rapidly that the reorganizing cells kept in close contact with the original sporogenous cell walls. Under suitable stain, this investment of diffuse opaque material about the spore mother protoplast was seen to be very thick, and it was much laminated. Subsequent to the amoeboid period (HAUPT **18**), the rounding and enlargement of the protoplast caused this mother wall to become tenuous.

Before the protoplasts had retracted very perceptibly from their sporogenous walls, the nuclei betrayed presynizetic activity (fig. 4). The stainable chromatin collected into a single body in the midst of a mass of indistinguishably intricate linin fibers, which stretched away from the nuclear membrane to which they seemed to be connected by many unbroken filae. Fig. 5 shows complete synizesis. The massing of the synizetic figure occurs, very commonly, opposite a thickened bed of cytoplasm, but not invariably so. Whenever the synizesis juxtaposes a thick border of the protoplast, a condition indicating some sort of polarity of the entire cell is made very evident. In fig. 5 the nucleolus is uppermost in the knot, where it is crowding the linin to the nuclear wall. It is seldom that the figure is seen in any other position, that is, in any position that cannot be accounted for by the vicissitudes of orientation in sectioning. When the synizetic skein unfolds, the nucleolus will retreat in advance, and will pass ultimately to (or nearly to) the opposite wall. This corresponds with HARPER'S (**17**) findings in Ascomycetes. The nearly spherical form and the remarkable size of the nucleolus at this stage provide a strong argument for the presence of some osmotic or retention membrane at its periphery. Its absorptive power is indicated by its chromatin storage function. The linin beneath it shows a circle of strands much coarser than the fibrils of fig. 4. There is considerable evidence that the continuity of the presynizetic network is being somewhat broken up by the contraction.

When the nucleolar retreat ensues (fig. 6), the linin is drawn toward the antipolar border of the nucleus. It maintains its connection with the polar nuclear border by means of persistent anastomosing threads. This incipient spireme displays the coarsely outlined stranding of fig. 5, and certain irregularities suggest the localization

of the chromatin and the presence of anastomoses. Aside from a slight decrease in the size of the nucleolus, at this stage there is little to suggest the conventional spireme; nor, in the present series of preparations, has any evidence been furnished that a true synapsis of duplicate spiremes has occurred at any period.

The recovery from synizesis often displayed a course somewhat different from the behavior depicted in fig. 6, in that the linin fibers show more distinctly. The thread may be smoother, more definitely contoured, more chromatic; and it may show even more plainly its filar anastomoses with the nuclear wall. The nucleolus, however, will have diminished markedly in size (fig. 7), and it will show broad trails down which the chromatin seems to be passing to the linin reticulum. The central mass of the nucleolus, however, generally displays smoothness of contour. In fig. 8 the nucleolus has been bisected, each portion carrying its own quota of the spireme. This is as extensive separation of the nucleolar elements as would commonly occur, since by far the majority of instances show the nucleolus as a single body. Fig. 9 shows an unequal nucleolar division, attended by an inequality in the distribution of the spireme. This latter structure has lost the slightly beaded appearance of fig. 8, and it has diminished in number of internal strands, but peripherally there appear the cut ends of other strands abutting the nuclear membrane. How intimate may be the association of spireme and confining nuclear membrane is difficult to determine. This section was cut transversely to the polar axis of prophase.

Whether the nucleolus remains solitary, double, or quadruple in mid-prophase, and whether the spireme shows marked disassociation or not, there is always a very marked diminution in the amount of chromatin remaining visible within the confines of the nucleus. It is not thought that the linin threads of the spireme actually disappear, but their inability to retain stain causes them to simulate such disappearance. Such a suggestion is given by fig. 7, where little superficial chromatin remains on a spireme that has become a weft of linin fibrillae with conspicuous attachment to the nuclear border.

The cell now shows a period of rapid growth, and the intake of nucleoplasm may account for the irregularities soon supervening in the linin association. Without losing its connection with the central

chromatic mass, it becomes a diffuse tangle of threads that course erratically throughout the nuclear cavity (fig. 10). The figure is a presentation detailing nuclear phenomena as faithfully as possible. The disappearance of the chromatin is a problem, since the linin fibers are not visibly increased in number or in staining capacity. If it is resolved into karyolymph, this would account only partially for the marked nuclear enlargement. A marked increase which now supervenes in the staining power of the cytoplasm could hardly be correlated with the dissolution of the chromatin, unless there is partial exosmosis of the latter substance. This loss of visibly staining chromatin from the nucleus, however, is final and relatively complete. The ultimate breaking up of the nucleolar mass results in numerous very dense elements imbedded in an opaque matrix which is the source of the radiating meshes of the linin structure. The latter is so much reduced that it can hardly be thought of longer as a spireme.

As the cell approaches very closely to the actual reduction process, it becomes increasingly evident that not the linin spireme but the nucleolar body is the significant factor in the chromatic history of the cell. There is no indication of a segmenting spireme in the usual cytological sense, but there has been increasing evidence that surplus chromatin is being passed along to other places, the linin thread being but one of the means of translocation. Figs. 11–13 show that chromatin elision carried to its maximum extent leaves a residue, the metaphase chromosomes. Contrasted with the huge nucleolus of synizesis, the chromosomes are incredibly small, and they seem to emerge from nodal or specially localized points in the nucleolar mass.

At this period certain significant phenomena have occurred in the cytoplasm also. The early, densely granulose protoplasm has become a grossly vacuolated, mature cytoplast, composed of coarse radiating wefts of finer fibrillae. The plastids dispersed in its meshes are usually rendered invisible by the chromic fixation. Furthermore, great vacuoles in the protoplast cut out a dense layer of cytoplasm that invests the nucleus closely. This region finally becomes so reduced in thickness by the expansion of the nucleus that it becomes little more than an investment where all the peripheral strands of the

cytoplast terminate centrally. No evidence could be established that this zone constitutes a specialization in the form of kinoplasm, but it certainly represents the region in which kinoplasm would be expected.

The wefts of the cytoplast are radiating, infrequently anastomosing paths between two very significant regions, the periphery of the nucleus and the periphery of the cell. The exterior limiting layer has already functioned in laying down a protective wall, to which it is closely adherent unless disturbed by some extraneous interference. It is active as a secretory region, and undoubtedly has much control over nutritional elements entering from the carpocephalic tissues. It is a medium for a vacuum-like support of the protoplast, tending to prevent collapse with its attendant injury to the fine internal mechanism that is in control of cell metabolism. It is then not inconceivable that the inner layer investing the nucleus is also just as significant and possesses just as much control over the activities of the more central regions of the cell. Externally, a membrane was elaborated. Internally, there might appear (at a slightly different period of ontogeny) a slightly altered substance, kinoplasm, differing from the nuclear membrane itself mainly in the matter of continuity and cohesion. This secretory function conceivably might lie dormant, awaiting appropriate stimulus from some element not apparent as yet.

Shortly subsequent to synizesis, there appear within the nucleus certain bodies unfamiliar to the literature of reduction division. For convenience of terminology, they may be designated as nuclear bodies, since their activities are confined largely to that region of the cell. As their presence in the last sporogenous cells has not yet been established, for the purposes of this study it may be said that they arise during the early prophase of reduction division.

In character they are constantly small, homogeneous, brilliantly staining corpuscles; they are smooth surfaced, and apparently are inclosed in a thin pellicle of like character with the linin. This investment is hard to detect except under favorable differentiation. When seen it is quite as definite as the pellicle of a young plastid. What their origin might be was hard to determine; but the *locus* of their appearance usually was the region of the synizetic knot, sub-

sequent to some considerable alteration in the size, form, continuity, or position of the nucleolus. They cannot be interpreted casually as mere fragments of the nucleolus, as they appear long before that structure has resolved itself into granules approaching anything like a similar size. To follow their history serial sections are obligatory; sections 12–20 μ thick are best in very late prophase.

As a rule, the nucleolar bodies take the same stain as does the nucleolus. Under heavy counterstain they sometimes emerge in a contrasting hue, this phenomenon being due, undoubtedly, to the selectivity of their hyaline investment. Their apparently inconstant features are relative position, degree of prominence, and something of a variation in size. Very frequently one or both may be removed in sectioning.

The first appearance of the nuclear bodies was at the polar margin of the nucleus, and at the base of the unfolding spireme. In this obscure position they are inconspicuous; in later stages they are revealed prominently (fig. 7). Fig. 8 shows them obscured within the loops of the spireme. The nuclear bodies leave the synizetic knot very early, and pass to more or less opposite regions of the nuclear cavity (fig. 7), carrying with them certain attached aggregations of the linin. Sometimes they show discrepancy of size, but usually there exists the uniformity displayed in fig. 7; and especially is this the case in the stages nearing metaphase, when the mother cell has probably reached its maximum prereduction dimensions. Unless plasmolyzed, the nucleus has become so large and so symmetrically round that the cell presents a very striking appearance. After this period the nucleus begins to show irregularity, and its membrane becomes more difficult to detect. The nuclear bodies of the polar region are connected by linin threads to the remnants of the nucleolus, out of which are developing the chromosomes in the antipolar region of the nucleus (fig. 3).

When the bodies arrive at the exterior margin of the nucleus, they attach themselves to the nuclear membrane. A fluff or haze of a peculiar type begins to surround them, and it involves the adjacent cytoplasm. It appeared to be quite certain that the nuclear membrane disappeared first in their vicinity; the remainder passed away gradually. The nuclear angle in which the chromosome com-

plex rests is not an especially active center, as is evidenced in fig. 12; while the regions in which the nuclear bodies lie are most especially so. Sometimes the bodies are not so remote from each other, but they proceed to separate (fig. 11) until they establish a polarity of the protoplast diametrically opposed to the initial nuclear polarity. In the process the nucleus becomes very sharply angled at the points at which the nuclear bodies occur (fig. 11), while the remaining regions depart more slowly from the original rounded form. These changes result in a structure somewhat comparable with the early quadripolar figure so much discussed in the Jungermanniales. Any degree of plasmolysis at this period enhances the quadripolar effect by sharpening the natural outpocketings of the nuclear cavity.

When the nuclear bodies have access to the cytoplasm after the destruction of the nuclear membrane, they appear to become ensconced in its meshes, and they may become the center of an indefinite granular mass. Figs. 11–13 show a somewhat definite sequence in the behavior of these bodies as they exist in the border of the cytoplasm, and as they continue to move or to be drawn apart. Fig. 12 especially shows the major portion of the nuclear wall destroyed, and it portrays also the peculiar thickening and shaping of the cytoplasmic reticulum (upper pole) around the migrating nuclear body. The opposite pole was sectioned too close to the related body to show any special cytoplasmic aggregation.

HETEROTYPIC SPINDLE

The actual rise of the spindle was not demonstrable; but a prolonged checking up of data item by item, for previous and for subsequent periods, compelled the belief that these are the initial visible phases in the rise of the heterotypic spindle. This belief is fortified further (fig. 12) by the presence of the peculiar granulations that appear to the left of the chromosome group; these are thought to be the transected fibers of an early amphiaster. This would mean that spindle fibers begin to appear before the poles are diametrically opposite. Thus the earliest form of the spindle would be triangular, the poles developing from the polar nuclear regions, while the third angle contained the less active chromosome complex of the antipolar region.

Fig. 13 shows the spindle with a straighter axis, a vanished nuclear membrane, and a further emergence of the chromosomes. Although spindle fibers are not demonstrable, it is felt that they are being developed rapidly. The upper pole of fig. 12 has pushed farther into the cytoplasm, encysting itself, and great wefts of the cytoplasm are organized about it. The impression is gained that the poles are constricted cylinders of tough thick fibers mutually supporting and being supported by the jacketing cytoplasmic reticulum.

Difficulty was encountered in demonstrating certain continuous peripheral fibers which lay far outside the zone of the chromosomes in the metaphase plate. In fig. 14 they were secured by staining heavily with haematoxylin, with total omission of all destaining. It is thought that their relationship to the metaphase plate might indicate that some of these rather coarse fibers might have been derived from the nuclear membrane. At a later stage it is certain that other fibers, which end freely in the peripheral cytoplasm, come to lie exterior to these fibers. In fig. 14 the underlying equatorial plate, seen in profile, is so compact that the individual chromosomes cannot be distinguished.

The spindle is a structure of progressive development. Likewise its activities are discontinued, and its architecture is remodeled or dismantled just as gradually. In every direction from the pole, peripheral fibers continue to be sent to the outlying cytoplasm. They are not (as they have appeared to many observers) mere aggregations of the ordinary cytoplasm of the cell, but are gossamer strands inconceivably tenuous and smooth. They cannot be detected until they are present in abundance, and they are most visible *en masse*. Starting from a common center at the poles, numerous coarse fibers are seen to be enmeshed among much finer fibers. With regional restrictions, these latter permeate the protoplast, ending quite demonstrably in some strand of cytoplasm, a granule, or a plastid. These structures are definite asters; but they are so dense a complex of cytoplasm, kinoplasm, and trophoplasm that all attempts to detect the presence of any possible central body have proved fruitless. Such a structure as is shown in fig. 12 was too indefinitely delineated to warrant its classification as a central body. That these regions are a complex of differing protoplasmic gels is shown by the fact that

the spindle fibers and asters commonly take the violet tone of FLEMMING'S triple stain, while cytoplasm and granulose kinoplasm are distinctly roseate under the same treatment.

There is little evidence for the presence of distinct mantle fibers, but there are many central fibers in the newly formed spindle. It is upon the outer fibers of this group that the chromosomes seem to be borne; indeed fig. 15 would indicate their presence throughout the group. Many times the chromosomes are so obscured that they would seem to be attached to the inner faces of the fibers upon which they are adherent. In anaphase it is these connecting fibers which transect, and which carry the chromosomes poleward. Such fibers of the group as do not bear chromosomes then develop, apparently, the usual double equatorial line of granular swellings that constitute the early cell plate.

The completed heterotypic spindle occupies the former nuclear cavity in such a way that it constitutes an intra-nuclear spindle, with its poles only imbedded in the cytoplasm. During the metaphase the spindle begins a period of elongation that so stretches the outlying cytoplast that it comes to envelop the spindle closely. The first indication of true metaphasic activity is a lateral extension of this taut spindle. The fibers spin outward at the equator until the densely packed chromosomes begin to separate laterally from one another. As the chromosomes separate, they appear (fig. 15) as 16 small, blunt rodlets, the bivalent chromosomes. No indication of the homotypic splitting exists in metaphase. Having been spread apart by the spindle fibers, the bivalent chromosomes continue to separate from one another (fig. 16) without displaying tetrad characters. Since in anaphase (fig. 17) more than 8 bodies pass toward each pole (14 having actually been counted), it is evident that the homotypic split, if present, is fully concealed. There appears to be no evidence either for or against telosynapsis. In late anaphase the polar group is very dense (fig. 18), and as it passes into telophase it is drawn very far toward the cell's periphery. When the kinetic pull is relaxed, and when reconstruction supervenes, the daughter nuclei settle back toward the former nuclear border.

From the time of the inception of the metaphase, the connecting fibers of the spindle have steadily spread outward in the equatorial

region, enlarging what was originally the nuclear cavity. At first this orbicular space is bounded by the spindle fibers which had pressed back the cytoplasm. The phragmoplast thus created probably incloses some karyolymph; it is most certain that the interzonal spindle fibers have contributed to the formation of an incipient cell plate traversing the phragmoplast cavity. The spindle fibers disappear, but the cell plate (fig. 19) persists. No evidence was afforded of the presence of a cellulose septum within it. Undoubtedly it is the first cleavage plane in the cell, but its complete functioning is delayed until after the time of the homotypic mitoses. Also the many peripheral fibers of the first spindle may have operated to extend the cleavage impulse beyond the confines of the phragmoplast, which evidently had terminated its possible fulfillment through the agency of the central spindle fibers.

Homotypic spindle

The interkinesis is brief, but the daughter nuclei approximate a condition of rest. The telophase group of fig. 18 becomes the object of a new diminution of chromatic matter. Nuclear and cell growth continue, and the cell may elongate very slightly before the final karyokinesis. The first hint of a membrane around the reorganizing nuclei appears as a thin blue halo whose borders follow the irregularities of the dense cytoplasmic wefts that held the heterotypic poles in position. The red staining chromatic body is inclosed. Small, deeply staining spherical bodies appear within the halo, and they usually keep in close contact within its borders. The chromatin masses break apart (fig. 19) and separate slightly. As they enlarge, the nuclei lose their irregularity; and if they have migrated far peripherally, they begin their retreat to the border of the persistent phragmoplast cavity. The nuclear chromatin then breaks up into successively smaller bodies, until it is well distributed upon a very evident slender linin reticulum. Several chromatin masses persist.

As the homotypic metaphase approaches, the nuclear bodies migrate to four distinct points upon the border of the former nuclear (now the enlarged phragmoplast) cavity. The nuclei elongate (fig. 20) as the nuclear membranes are fading, and this constitutes added evidence that the nuclear bodies free themselves from their im-

mediate nuclear confines, and follow along the kinoplasmic tracks derived from the disorganization of the peripheral fibers of the previous karyokinesis. What forces determine the axes and the terminal points of this migration is a feature of the cell's behavior that is not easily comprehended. Again, the nuclear bodies could not be identified with the poles of the spindles, which appeared more swiftly than had their predecessor, owing to the abundance of potential kinoplasm residuary from the heterotypic asters.

The homotypic spindles may be parallel, but usually they are opposed. Their formation draws or distorts the cavity of the phragmoplast, since they organize in part from the kinoplasmic substance of its borders (fig. 20). Slightly curved at first, they commonly straighten in anaphase because of the tenacious hold of their poles, which have become knitted solidly into the cytoplasm by means of their astral rays. With the exception of size, these asters are the duplicates of the asters of the first karyokinesis.

Sixteen bodies appear in the metaphase plate (fig. 21) which are interpreted as univalent chromosomes, since more than eight very small bodies pass to each pole in anaphase. At the poles the chromosomes collect into a ball as before, and the spindles elongate, thrusting the masses of chromatin well into the cytoplasm. Profuse peripheral spindle fibers again spread outward from each pole in a wide cone.

The extreme thrust of the telophase carries the four new nuclei to the periphery of the cell. Reorganization of the granddaughter nuclei begins almost at once, and its inception is evident long before the cleavage has appeared that actually divides the spore mother protoplast into four parts. As the nuclei retreat, a slight peripheral film again appears which becomes a rim or nuclear membrane whose irregularities straighten out and again inclose the diminutive nuclear bodies and the more perceptible masses of the chromatin. The latter break up into progressively smaller bodies, any residue becoming a new nucleolus bathed by an abundance of karyolymph. Finally, delicate anastomosing threads are visible that traverse the whole nucleus (fig. 23). With the completion of the quartering of the protoplast, the spore nuclei will have attained a normal spherical turgid-

ity, will be centrally located, and will not differ appreciably in size from the last sporogenous nuclei.

The present study does not support the view that the spore mother protoplast is divided by means of surface cleavage furrows. Both homotypic spindles complete their telophases by a peripheral extension of spindle fibers. Subsequent cell plates initiate two secondary cleavage planes which are opposed to the primary cell plate. While cleavage is effectual first at the center of the spore mother cell (fig. 22), it operates simultaneously and equally in all planes (fig. 24). The *modus operandi* is the appearance within the cytoplasmic strands of a duplicate bed of granulations that ultimately flow together to form cytoplasmic films, the plasma membranes. Later these are separated from each other entirely, by the severance of any remaining plasmodesmen. Stains afford no evidence of the presence of an intercalated cellulose wall (fig. 24) at this early stage.

Subsequently the segregated spores display denser cytoplasm just within the new plasma membranes; it becomes a very dense zone in which are held plastid primordia and other granules. The activity of wall secretion is begun previous to any very perceptible rounding of the protoplasts. These latter may not be in close contact centrally if the cavity of the phragmoplast has been large and persistent; but the rapid increase in cell turgor quickly obliterates this space, and the tetraplasts acquire ternate faces interiorly, and a convex surface exteriorly. The dense layers of cytoplasm just beneath the plasma membrane contain plastids in abundance, and they become much larger at this period. Oil globules and other metaplasmic granules are abundant.

This thickening of the exterior portion of each spore protoplast is so marked that it could be mistaken for an early manifestation of the new spore wall. It cannot be asserted, however, that the spore coat has begun to form at any time previous to the appearance of a faint thread of contrasting color between adjacent protoplasts. Later this thread widens into a broad opaque band which stains densely, and which thickens with equal rapidity between the ternate faces of the maturing spores, as well as on their exterior borders just beneath the mother wall (figs. 24, 25).

In fig. 26 the exospore is splitting. The exinium hugs the protoplast, while the perinium bulges marginally, preparatory to the growth of the basal flanges of the mature spore. Exteriorly (fig. 27) the perinium is thrown up into exaggerated excresences which are most marked on the outer convex surface of the spore. The underlying exinium shows numerous filar connections with the perinium. The thinner intine hugs the protoplast, and the underlying plasma membrane is very thin. The cytoplasm is replete with nutritive inclusions. The spore has increased in size enormously subsequent to the reduction division. This is shown in fig. 28, in which is depicted a normally ejected mature spore that has been reduced to one-fourth of the scale of enlargement that was established for this series of drawings.

Summary

1. The premeiotic protoplast shrinks away from its angular sporogenous walls. Within the space created it secretes a special external membrane that functions as the wall of the spore mother cell.

2. A marked contraction of the nuclear network is preliminary to the reduction division. This synizetic knot displays a large, orbicular, deeply staining nucleolus that rests at the polar border of the nucleus.

3. The nucleolus retreats from the polarized nuclear border toward an opposite antipolar region in the nucleus. Anastomosing freely with the nuclear membrane, the spireme recovers as coarse, looped strands centered in the nucleolus or in its disassociated parts.

4. There is no evidence of the pairing of spiremes, nor that the post-synizetic spireme is a continuous thread. Suffering constant elimination of chromatin, it is reduced to slender irregular strands.

5. The chromosomes are short thick rods. They are not derived by the segmenting of a visibly synaptic spireme, but arise as localized masses in the large chromatin nucleolus.

6. The sixteen bivalent chromosomes appear upon the early spindle in juxtaposition. They are disassociated in metakinesis upon reaching the equatorial plate stage. They show no evidence of tetrad structure, but split apart evenly upon leaving the metaphase plate; sixteen chromosomes arrive at each pole.

7. Reduction occurs by means of one heterotypic spindle succeeded by two simultaneous homotypic spindles that usually lie in opposed planes.

8. The homotypic metaphase plates show sixteen chromosomes; and as more than eight chromatids pass to each pole, the gametophyte is considered as having sixteen chromosomes.

9. An incomplete cell plate follows the heterotypic spindle. Further cleavage is deferred until the time of the homotypic telophase, when secondary cell plates cooperate in effecting a simultaneous cleavage of the mother protoplast in three directions. All originate as duplicate thickenings of the central spindle fibers.

10. Externally to the spindles, cytoplasmic rearrangement is continued under the control of peripheral spindle fibers. Cleavage proceeds centrifugally.

11. No intercalated septum occurs previous to general spore wall deposition. No ingrowth of the spore mother cell wall occurs, nor any furrowing in the periphery of the protoplast.

12. The spore coats are laid down in centripetal succession by the activity of the plasma membranes. The spores are freed by the rupture of the attenuated spore mother cell wall.

13. No specialized zone of kinoplasm, nor any hyaline polar caps were seen. The spindles possessed true asters.

14. Small intranuclear bodies seemed to have significance during the early stages of spindle formation. They showed no evidence of a centrosomic nature.

The writer is much indebted to the late Professor WILLIAM L. WOODBURN for valuable suggestions and criticisms in the development of this investigation.

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EXPLANATION OF PLATES XXX, XXXI

In the execution of these drawings a Bausch & Lomb 1.9 oil-immersion objective was used with a Huyghenian ocular 10. A camera lucida was employed at table level, with arm at 100, and draw tube of microscope at 160 mm. With the few exceptions noted, all figures were magnified 912 diameters. The plates were reduced two-fifths in reproduction.

PLATE XXX

FIG. 4.—Last sporogenous telophase, showing amoeboid protoplast retreating from tenuous cell wall.

FIG. 5.—Synizesis occurring at polar border of nucleus.

FIG. 6.—Recovery from synizesis by retreat of nucleolus from polar border of nucleus.

FIG. 7.—Antipolar position of nucleolus, showing chromatic linin strands attached to polar border of nucleus.

FIG. 8.—Early bisection of nucleolus, with linin associations attached to each segment.

FIG. 9.—Older stage; transverse view showing transected chromatin threads on nuclear border.

FIG. 10.—Nuclear details in section 10 mm. thick, showing attachment of linin thread to eroded nucleolus, nuclear border, and nuclear body: ×1140.

FIG. 11.—Orbicular nucleus changed to quadripolar figure, antipolar view; nuclear bodies situated in sharpened angles, with chromosomes lying at lower level.

FIG. 12.—Composition study showing adjacent astral and nuclear sections; cytoplasm being incorporated into astral complex; transected interzonal fibers at left of chromosome group; nuclear membrane still persists at right; nuclear body below.

FIG. 13.—Spindle axis established in one plane; nuclear bodies antipodal; nuclear membrane gone.

FIG. 14.—Heterotypic spindle whose peripheral connecting fibers wholly inclose equatorial plate.

FIG. 15.—Polar view of heterotypic metaphase plate.

FIG. 16.—Lateral view of very early heterotypic anaphase.

FIG. 17.—Later anaphase.

PLATE XXXI

FIG. 18.—Telophase showing thickening of spindle fibers in equatorial region.

FIG. 19.—Reorganizing daughter nuclei retreating from periphery of protoplast; phragmoplast cavity shows incipient cell plate.

FIG. 20.—Elongation of daughter nuclei indicating initial stages in formation of homotypic spindles.

FIG. 21.—Homotypic division showing opposed spindles; it presents one homotypic equatorial plate in polar view.

FIG. 22.—Completed homotypic anaphase, showing early nuclear phases of three associated spores; cleavage plane of first mitosis still visible.

FIG. 23.—Very late telophase, or formation of spore nuclei; cell plate significant of future cleavage lines.

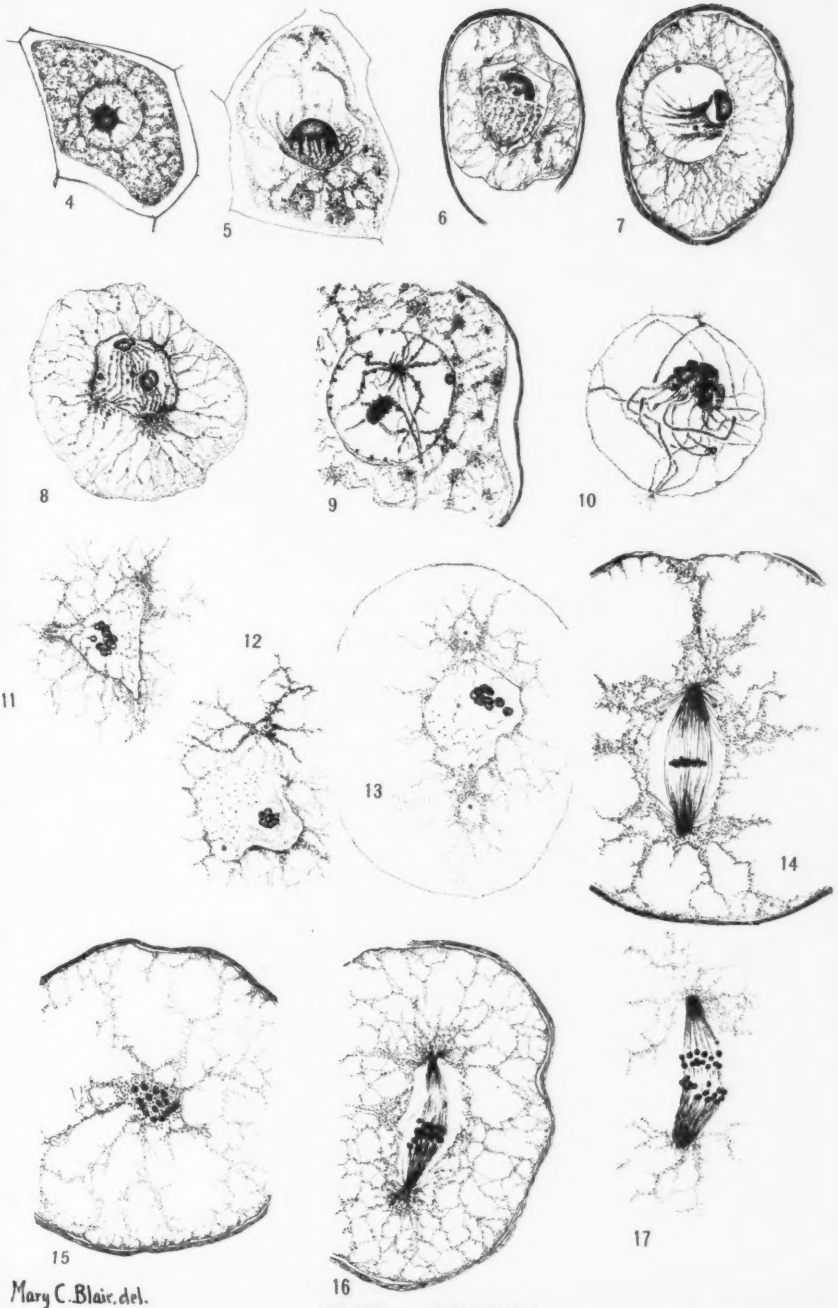
FIG. 24.—Early cleavage of mother protoplast, showing formation and progress of plasma membranes.

FIG. 25.—Exospore thickening around each member of tetrad; ×408.

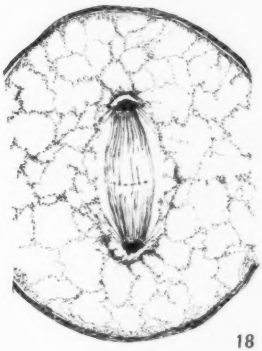
FIG. 26.—Exospore splitting into perinium and exinium; mother wall shown as ruptured remnant; ×408.

FIG. 27.—Section of mature spore from black sporophyte just previous to rupturing of capsule; perinium folded; exinium thick; intinium invests plasma membrane; ×408.

FIG. 28.—Outer face of ejected spore showing reticulations and marginal rim; ×207.



BLAIR on REBOULIA



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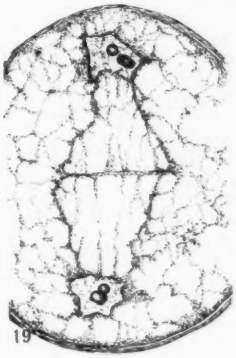

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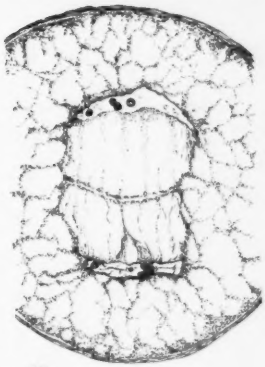

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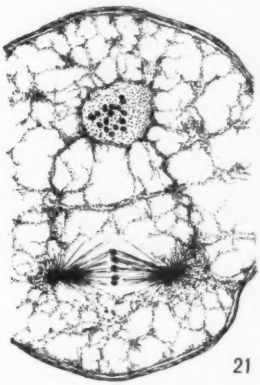

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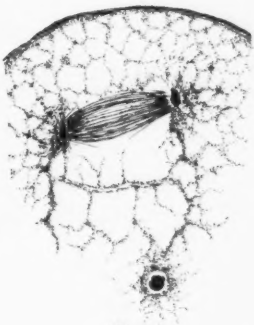

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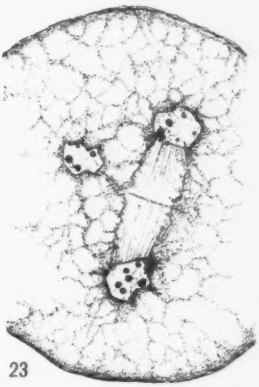

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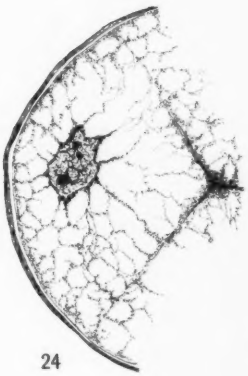

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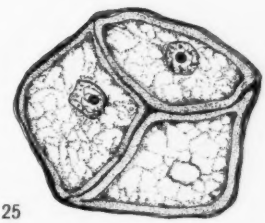

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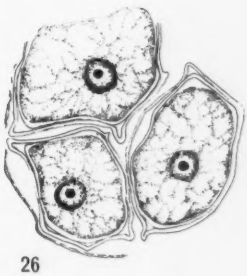

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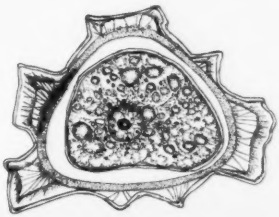

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Mary C. Blair, del.

BLAIR on REBOULIA

HYBRIDS IN CYCADS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 348

CHARLES J. CHAMBERLAIN

(WITH THIRTEEN FIGURES)

It is doubtful whether any true hybrids in cycads have ever been described, although there is a note on hybrid cycads[1] dating back as far as 1883. In FOCKE'S *Pflanzen-Mischlinge* (1881), all the principal reputed plant hybrids are listed in systematic order, but no cycads appear on the list. He makes the statement, however, that the pollen of cycads "has occasionally been employed to fertilise the female of other species; but I am unaware whether perfect seeds have been obtained or hybrids raised."

In the note on hybrid cycads, HEMSLEY states as follows:

> Mr. KATZER, chief of the Imperial Gardens at Paullowsk, near St. Petersburg, had succeeded in raising a hybrid between *Ceratozamia longifolia* and *C. mexicana*. In this instance, a male of *C. longifolia* first developed an inflorescence, and Mr. KATZER collected the pollen and preserved it in a sealed bottle until a plant of *C. mexicana* produced a cone. When the flowers of the latter were in a receptive condition they were sprinkled with the pollen of *C. longifolia*, resulting in the development of perfect seeds, some of which germinated last year (1882).

These were claimed to be the first hybrid cycads; but as soon as this claim appeared, it was found that WEILBACH, Curator of the Botanical Garden at Copenhagen, had fertilized *Ceratozamia robusta* Miq. with the pollen of *C. brevifrons* Miq., and obtained seeds which germinated. These hybrids were sent to various gardens. MIQUEL himself determined the species of the parents; but WARMING later identified the so-called *C. robusta* as *C. longifolia*, and *C. brevifrons* as *C. robusta*.

HEMSLEY remarks:

> There is only one thing that is certain in all this, and that is the uncertainty of the so-called species. It is quite possible, and indeed very probable, that we have here in both instances to do with the male and female of the same species.

[1] HEMSLEY, WM. B., Hybrid cycads. Gardeners Chronicle **19**:466–467. 1883.

A study of *Ceratozamia* in the field and in the greenhouse for a period of twenty years enables me to state with confidence that the uncertainty in those determinations is even greater than HEMSLEY intimated. DECANDOLLE, in his *Prodromus*, lists *C. longifolia* and *C. robusta* under *species dubiae*. They are more than doubtful. It is safe to say that seeds of a single cone of *C. mexicana* could be made to yield plants of *C. mexicana*, *C. longifolia*, *C. robusta*, *C. Miqueliana*, and *C. brevifrons;* and this result could be brought about by raising the plants in containers of various sizes and by planting in the ground in various conditions. The different characters could probably be developed in less than twenty years.

My principal study of *Ceratozamia* in the field was made near Jalapa, Mexico, on the steep, well drained mountain side opposite the extinct crater of Naolinco. All the plants seemed to be typical *C. mexicana*. Plants raised from seeds collected here and planted at the University of Chicago were easily diagnosed as *C. mexicana;* but after fifteen years many of them had become *C. longifolia*. None appear to be *C. brevifrons*, but that may be due to the fact that none of them were kept in small containers. *C. Küsteriana* may be a good species, but it looks as if the rest could be raised from *C. mexicana*.

The pollen of cycads is short lived. It gives the greatest percentage of germination when first shed; after a few days there is a notable decrease in the number of spores which germinate; at the end of ten days less than half the spores germinate; at the end of three weeks very few spores germinate; and a month from the time the pollen has been shed I have not been able to germinate the pollen of any cycad. Mrs. ALICE BAILEY, who is particularly skillful with culture media, was able to germinate pollen a few days after my attempts failed, but a month can be regarded as the limit of life of *Ceratozamia* pollen.

This would mean that while KATZER went through the motions of pollination, it is practically certain that there was no fertilization. That embryos were developed and seeds obtained I do not doubt; but it is well known that in *Ceratozamia* and *Encephalartos* the female cones and their seeds develop to full size, even without any pollination. SEDGWICK[2] proved conclusively that *Encephalartos* sometimes

[2] SEDGWICK, P. G., Life history of *Encephalartos*. BOT. GAZ. 77:300–310. 1924.

produces embryos without any pollination. There is, in this case, a fusion of the ventral canal nucleus with the egg nucleus, just as one finds it occasionally in *Pinus*, *Picea*, and *Ginkgo*. It is practically certain that KATZER'S so-called hybrids were of this type, or at least were produced without fertilization by sperms. To summarize, it may be stated, as it was said in 1851 and 1882, that the plants here described are the first hybrid cycads.

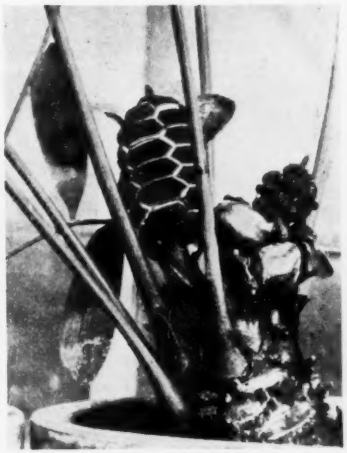

FIG. 1.—*Zamia latifoliolata:* cone at right, showing two of the ripe seeds, pollinated with *Z. pumila;* cone at left pollinated a year later with *Z. floridana.*

Even in limited collections in greenhouses there are occasional opportunities for crossing cycads. The pollination should be done just as the scales of the female cone have opened enough to allow the pollen to enter easily. When the opening between the scales is at its maximum, the pollination drop at the end of the micropyle is in the best condition for receiving the pollen. If pollen is abundant, it may be placed on a sheet of paper and puffed against the female cone; but if the pollen is from a small cone, like that of *Zamia latifoliolata*, a method devised by our head gardener is better. He takes a long glass tube, with one end drawn out like the tip of a pipette, touches some pollen with this tip and blows it into the crevices between the scales.

During the past five years several cross pollinations have been made in the University of Chicago greenhouse, most of them between different species of the same genus, but some of them between different genera. With the parents and the F_1 generation well labeled, it may be possible, within the next ten years to pollinate intelligently for an F_2 generation.

Zamia latifoliolata×Z. pumila

On December 20, 1921, I pollinated the female cones of two plants of *Zamia latifoliolata* with pollen of *Z. floridana*. The *Z. latifoliolata* was brought from Porto Rico, and the *Z. floridana* was secured from Miami, Florida. The scales of the female cone were

FIG. 2.—*Z. latifoliolata*×*Z. pumila*, F_1 generation

tightly closed, and it is doubtful whether any of the pollen not washed off by spraying survived until the scales opened. A week afterward the scales of the female cones opened and both cones were pollinated with pollen from a plant of *Z. pumila* which had been secured at Hawks Park, Florida. One of the cones produced three good seeds and the other produced four, all of which were

planted and four of which germinated. The condition of one of these cones when the seeds were ripe is shown in fig. 1. The cone at the left in this figure is a year younger. Its scales are just beginning to open. Two or three days after this negative was made, several of the scales were open and the cone was pollinated (December 15,

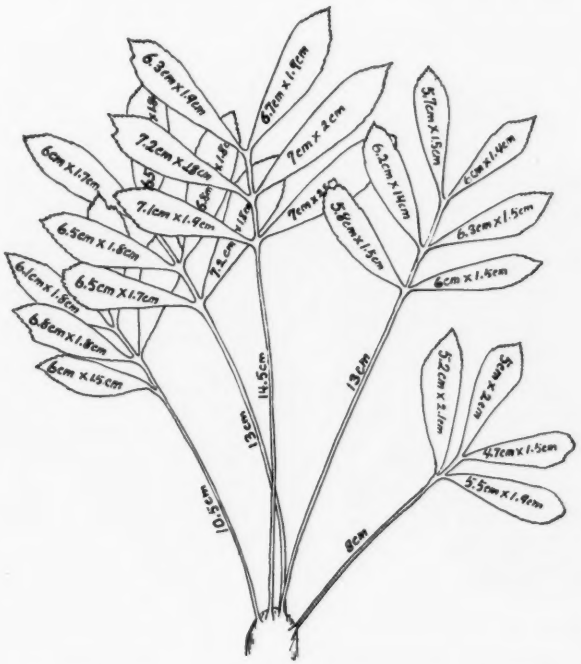

FIG. 3.—*Z. latifoliolata*×*Z. pumila*, showing size relations; one-half natural size

1922) with *Z. floridana*. This also seemed to be a successful pollination, for the cone grew, and on November 21, 1923, the seeds were ready to fall out.

The four seedlings resulting from pollination by *Z. pumila* grew vigorously, and on February 26, 1925, a little more than three years after the pollination (December 30, 1921), and two years after planting the seeds (January 9, 1923), they presented the appearance

shown in fig. 2. The size relations are shown somewhat diagrammatically in fig. 3.

It is evident that, in general habit and the character of the leaves, *Z. latifoliolata* is dominant, and that *Z. pumila*, which has comparatively small narrow leaflets, is recessive. The F_1 generation also resembles the dominant parent in the general contour of the leaflets, their serration, and in the number and branching of the veins.

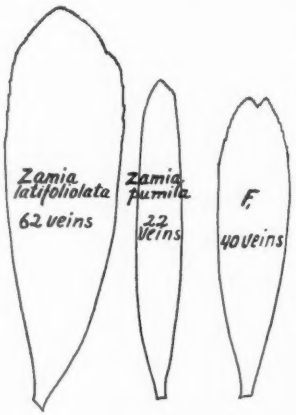

FIG. 4.—*Z. latifoliolata*×*Z. pumila*, leaflets of both parents and hybrid; one-half natural size.

The contour of the typical leaflets of *Z. latifoliolata*, *Z. pumila*, and their hybrid is shown in fig. 4. The veins are very numerous in *Z. latifoliolata*, often more than sixty; while in *Z. pumila* there are about twenty. In the F_1 generation the average number is about forty. We do not have seedlings of *Z. latifoliolata*, but four leaflets on a leaf developing from a bud scale had 33–40 veins. In the cycads such leaves resemble those of seedlings, or at least are juvenile in character. So it is probable that, so far as the number of veins is concerned, the F_1 generation closely resembles that of *Z. latifoliolata*. The histological character of the leaflet is also more like that of *Z. latifoliolata*, especially in the loose texture of the parenchyma between bundles.

If these four F_1 plants should reach the coning stage, as they may within the next ten years, and both sexes should be represented, and male and female cones should be produced at the same time, it would be interesting to see what would appear in the F_2 generation. Since the cones of the two parents are as distinct as their leaves, another pair of characters could be contrasted even before the F_2 generation is secured. Although *Z. latifoliolata* has a larger leaf, which is dominant in the hybrid, *Z. pumila* has a larger stem and larger cones.

Zamia latifoliolata×Z. floridana

The cone of *Z. latifoliolata*, shown at the left in fig. 1, was pollinated December 15, 1922, with pollen from *Z. floridana*. The seeds were planted November 25, 1923, and four seedlings survived. Their appearance on February 23, 1925, is shown in fig. 5 and in the somewhat diagrammatic fig. 6, which records details of size relations.

FIG. 5.—*Z. latifoliolata*×*Z. floridana*, F_1 generation

Here again it is evident that *Z. latifoliolata* is dominant and that *Z. floridana* is recessive. The seedlings look very much like those of *Z. latifoliolata*×*Z. pumila;* but it must be remembered that the female in this case is not only of the same species as that of the first hybrid described, but that it is the same plant. Besides, *Z. pumila* and *Z. floridana* are very nearly related species. The leaflets have the contour, serration, and venation of *Z. latifoliolata*. Fortunately in this case there are seedlings of *Z. floridana* for comparison (fig. 7). Its long narrow leaflet, with comparatively little serration, contrasts

sharply with the broad leaflet of *Z. latifoliolata*. Even the F_1 seedling shows double the number of veins found in the adult leaflet of *Z. floridana*.

In foliage *Z. floridana* differs more widely from *Z. latifoliolata* than does *Z. pumila*, and in the histological structure of the leaf the two species can be distinguished easily. In transverse section, the

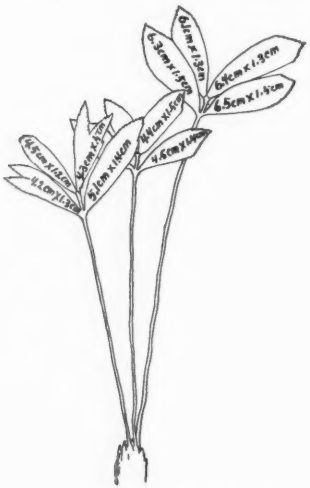

FIG. 6.—*Z. latifoliolata*×*Z. floridana*, habit sketch of F_1 showing size relations; one-half natural size.

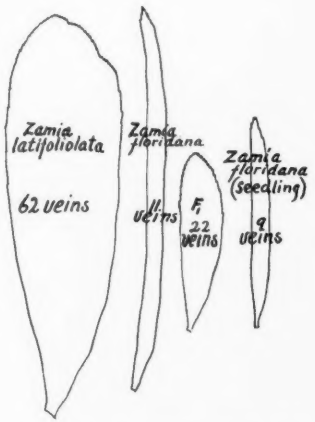

FIG. 7.—*Z. latifoliolata*×*Z. floridana*, leaflets of both parents and hybrid, also leaflet of *Z. floridana;* one-half natural size.

leaflet of *Z. latifoliolata* shows 6–8 suberized tracheids around the vascular bundle, while *Z. floridana* shows two or three times as many. The hypodermal layer of the abaxial side of the leaflet of *Z. floridana* is usually more or less sclerotic, while such a thickening is confined to the epidermis in *Z. latifoliolata*, except of course at the bundles, where all the cycads have sclerotic tissue.

The hybrid shows no sclerotic hypodermis on either side of the leaflet. The number of the suberized tracheids surrounding the bundle, as seen in the transverse section, varies from five to seven, while in the seedling leaflet of *Z. floridana* the number varies from eleven to fourteen. The mesophyll of the hybrid has large air spaces

like those of *Z. latifoliolata* and not at all like those of *Z. floridana*, in which this tissue is even more compact than in *Z. pumila*. In short, the foliage of the hybrid resembles that of *Z. latifoliolata*, both in topography and histological structure. Consequently, the dominant parent is the one with the larger leaves and broader leaflets.

FIG. 8.—*Z. pumila*×*Z. latifoliolata*, nearly mature cone

The cones and stems of *Z. floridana* are larger than those of *Z. latifoliolata*, but it will be about ten years before it will be known which parent is dominant in this respect.

Zamia pumila×Z. latifoliolata

On January 25, 1924, a female cone of *Zamia pumila* from Hawks Park, Florida, was pollinated by *Z. latifoliolata*. Only the upper

scales were open, but the cone grew to the usual size of cones of *Z. pumila,* and looked like a normal cone of this species (fig. 8). The first week in January 1925, the peduncle was accidentally broken, but the seeds were nearly ripe and the seed coats had an orange red color tinged with the darker red of *Z. latifoliolata,* rather than the light red with prominent orange which characterizes *Z. pumila.* The seeds were planted February 19, 1925, and several embryos broke the seed coat, but only one survived (fig. 9).

FIG. 9.—*Z. pumila*×*Z. latifoliolata,* F_1 generation.

The color of the seed coat and the general appearance of the seedling are those of *Z. latifoliolata;* so that in this case, as in the reciprocal cross *Z. latifoliolata*×*Z. pumila,* the *Z. latifoliolata* is dominant.

Zamia latifoliolata×Zamia monticola[3]

On March 15, 1924, a cone of *Zamia latifoliolata* was pollinated with *Z. monticola,* and four days later another cone was pollinated from the same male cone. The female cones grew to the usual size of cones of *Z. latifoliolata* and the seeds seemed to be normal. The color of the seed coat in the first cone was somewhat lighter and with more orange than in the typical cone of *Z. latifoliolata;* but in the second cone the seeds had the dark red color characteristic of *Z. latifoliolata,* with scarcely any tinge of orange.

The seeds were planted late in December 1924, but as yet no seedlings have appeared. Although the seeds have not decayed, it is doubtful whether any seedlings will be secured.

[3] New species of *Zamia* described in BOT. GAZ. **81**:218–227. 1926.

Ceratozamia mexicana × Zamia monticola

The most striking and successful cross made is one between *Ceratozamia mexicana* and *Zamia monticola*. One cone was pollinated March 23, 1924, with pollen from the same plant ot *Z. monticola* which had produced the cones used in all the previous pollinations in which this species was one of the parents. Another cone of *Ceratozamia* on another plant was pollinated on April 25, 1924, with pollen from another cone of that same plant of *Z. monticola*. This new species is very favorable for hybridizing, because, during the period described in this paper, it has coned every year, producing from four to six cones at a time. The cones appear in succession, and shed their pollen in succession. Of the six cones which appeared in 1920, the first began to shed pollen December 14, 1920, and the last pollen from the sixth cone was shed February 9, 1921. Since each cone continues to shed pollen for about a week, there was a period of nearly two months in which any female cone, which might open its scales, could be pollinated. I have not had under continuous observation any other cycad in which the period of shedding pollen was so prolonged, but there can be no doubt that in *Macrozamia Moorei*, with its 20–100 males cones developing in spiral succession, the shedding period is much longer.

From these two cones nearly a hundred seeds appeared to be well developed. They began to fall out naturally November 27, 1924, and during the next few days all were planted, except a few which were reserved for dissection.

Ceratozamia is unique among the cycads in having only one cotyledon, while all the rest have two. This fact was noted first by VAN TIEGHEM,[4] and later confirmed by WARMING,[5] MATTE,[6,7] Sister HELEN ANGELA,[8] and others. MATTE,[7] however, found two cases in

[4] VAN TIEGHEM, P., Symetrie des structures des plantes. Ann. Sci. Nat. V. **13**:204. 1873.

[5] WARMING, E., Ein Paar nachträgliche Notizen über die Entwickelung der Cycadéen. Bot. Zeit. **36**:737. 1878.

[6] MATTE, HENRI, Note preliminaire sur les germination des Cycadées. Rennes. 1907.

[7] ———, Memoires société Linnéene de Normandie. **23**:35–94. 1908.

[8] DORETY, Sister HELEN ANGELA, The embryo of *Ceratozamia;* a physiological study. BOT. GAZ. **45**:412–416. 1908.

which there were two cotyledons. VAN TIEGHEM'S observations were made upon four "hybrid" seedlings secured by pollinating *C. longifolia* with pollen from *C. mexicana* which had been shed three years before the female cone was pollinated. In three of the seedlings there was only one cotyledon, but the fourth seemed to have two very unequal cotyledons. In germinating a large number of seeds I never happened to notice more than one cotyledon. Sister HELEN ANGELA examined more than a hundred embryos and every one had only one cotyledon. In microtome sections for an anatomical study, however, she noticed occasionally a few tracheids opposite the cotyledon. *Ceratozamia* is unique among cycads in another feature, the seeds drop out of the cone soon after fertilization and before the various regions of the embryo have differentiated. Cones were secured from Mexico shortly before the time for the seeds to drop out, and Sister HELEN ANGELA fastened a great number of them to clinostats which were rotated until the embryos were mature. All of the embryos on the clinostats had two cotyledons; but checks, planted normally, had only one. This was conclusive evidence that *Ceratozamia*, phylogenetically, had two cotyledons, one of which had become suppressed.

If the seeds planted by MATTE (some of which were of my own collection from the same locality which produced the seeds studied by Sister HELEN ANGELA) were planted in any other position than on the side, I should expect them to develop two cotyledons. We always plant the seeds of cycads with the long axis parallel with the soil and nearly but not quite covered by the soil. More than sixty seeds planted in December 1924 germinated. Without disturbing the young seedlings, one could see that many of them had two cotyledons. In July 1925, when the seedlings were being repotted, a careful examination was made, and forty-seven of the fifty-six seedlings which had survived up to this stage showed two cotyledons; three had one cotyledon, and in the other six the cotyledon situation could not be determined without sacrificing the seedlings.

The two cotyledons are shown in fig. 10, and also in *A*, *B*, and *C* of fig. 11. In *C* the embryo has been removed from the endosperm. The development of the two cotyledons proceeds as in other cycads. In the usual single cotyledon of *Ceratozamia*, the outline of the

cotyledon is often somewhat C-shaped in transverse section, so that by looking at the open side of the C, one might imagine that there were two cotelydons; but a section of the hybrid embryo shows the

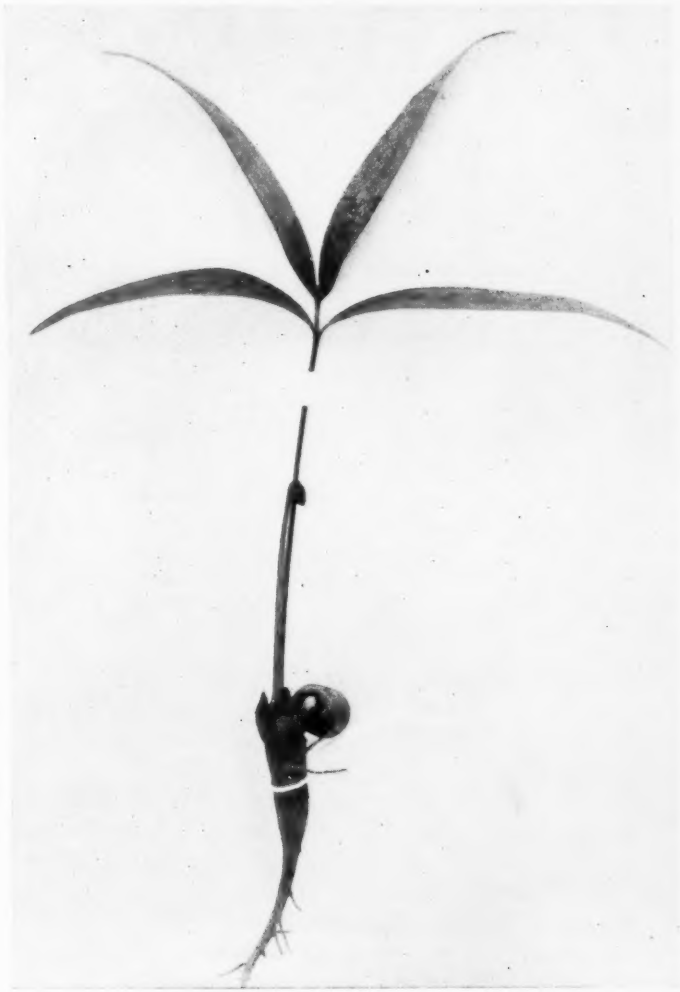

FIG. 10.—*Ceratozamia mexicana*×*Z. monticola*, F_1 generation

two cotyledons clearly, with the vascular supply just as described by Sister HELEN ANGELA for the dicotyl embryos developed on the clinostat.

One of the plants with a single cotyledon is shown in fig. 11 *D*. The stem apex lies between the leaf bud (*b*) and the cotyledon (*c*), but the suppression of the second cotyledon gives the leaf the position of the missing organ. Sections would show a few tracheids be-

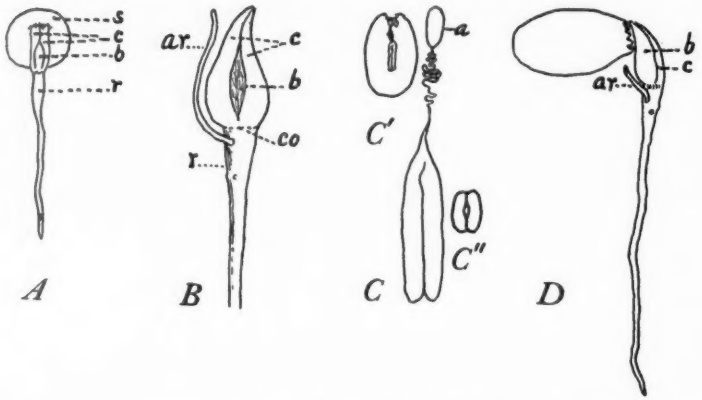

FIG. 11.—*Ceratozamia mexicana*×*Z. monticola*, F_1 seedlings: *s*, seed; *c*, cotyledons; *b*, bud; *r*, primary root; *ar*, apogeotropic root; *co*, coleorhiza; *a*, archegonium; *A*, *B*, and *C*, with two cotyledons; *D*, with only one; *C'* embryo in endosperm; *C''*, transverse section of embryo showing two cotyledons.

longing to the abortive cotyledon, so that the leaf bud is really between the two cotyledons. The apogeotropic roots, shown in *D* and also in *B*, are extremely common in cycads and are not at all the result of hybridization.

The leaflets of most of the seedlings are like those of *Ceratozamia* in contour, and all agree with *Ceratozamia* in having no serration; but many leaves of *Zamia monticola* have no serration. However, most of the leaflets are broad and have the contour of *Z. monticola*.

In this hybrid, *Zamia monticola*, the male parent is dominant in the character of the cotyledons and in most of the leaflets, while *Ceratozamia* is dominant in the general topography of the seedling and the contour of some of the leaflets. *Ceratozamia mexicana* has

a long slender leaflet tapering to a sharp point, and, like all species of *Ceratozamia*, if there is more than one species, entirely lacking in ser-

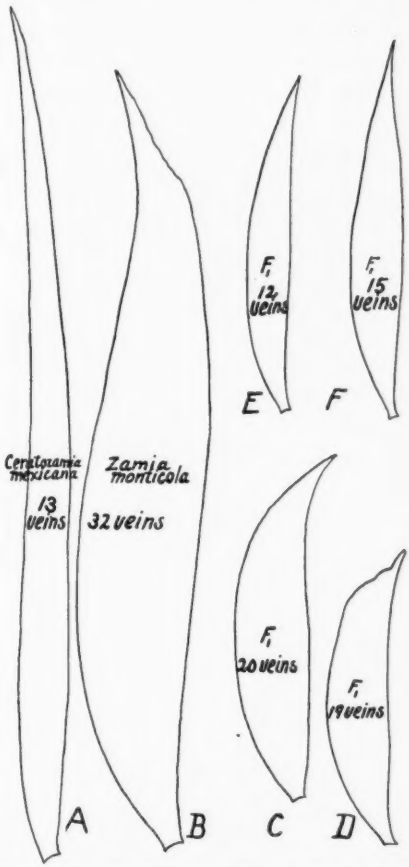

FIG. 12.—*Ceratozamia mexicana*×*Z. monticola: A, B,* leaflets of two parents; *C, D,* leaflets resembling those of *Z. monticola; E, F,* leaflets with contour of *Ceratozamia mexicana;* one-half natural size.

ration. In *Z. monticola* the leaflet is comparatively short and blunt at the apex. The contour of typical leaflets of the two parents and also leaflets of the hybrids are shown in fig. 12. In *C* and *D* of this

figure the leaflets have the contour of those of *Z. monticola*, and the number of veins is larger than in the adult leaflet of *Ceratozamia;* while in *E* and *F*, and also in fig. 11, the leaflets have the contour of those of *Ceratozamia*. The number of veins in leaflets of the *Ceratozamia* type, while smaller than in leaflets of the *Z. monticola* type, is nevertheless as large or larger than in adult plants of *Ceratozamia*.

FIG. 13.—*Z. pumila*×*Encephalartos villosus*, female cone shedding seeds

The histological structure of the leaflet of the hybrid resembles that of *Z. monticola* in the small number of suberized tracheids surrounding the bundle, and in the scarcity or entire lack of such tracheids between bundles. The difference here is very striking, because *Ceratozamia* has many such tracheids around the bundle and also between bundles. The mesophyll cells of the hybrid, in transverse section of the leaflet, are much elongated between bundles, while in *Ceratozamia* they are shorter and the tissue is more compact.

To summarize, the hybrid resembles *Z. monticola* in having two cotyledons and in the histological structure of the leaflet, as well as in the contour of most of the leaflets.

In this case both parents are large plants, with leaves more than a meter in length. *Ceratozamia* is arborescent, and this specimen of *Z. monticola*, a new species and the only specimen known, looks as if it might develop a trunk as large as that of *Ceratozamia*.

In the other crosses just described, all of them between different species of *Zamia*, both parents have small, tuberous, subterranean stems, and the parent with the greater display of foliage has been dominant in leaf characters.

In the cross between *Ceratozamia mexicana* and *Zamia monticola*, the latter is dominant in the characters of the leaf and cotyledons. The female cone of *Z. monticola* is not known, but the male cone, while very large for a *Zamia*, does not reach half the size of the average male cone of *Ceratozamia*. With about fifty hybrids now growing vigorously, it is quite possible that within nine or ten years there may be cones of both sexes, so that the cone character may be determined and an F_2 generation obtained.

Zamia pumila × Encephalartos villosus

On December 12, 1924, a female cone of *Zamia pumila* was pollinated with *Encephalartos villosus*. The *Zamia* was transplanted from Hawks Park, Florida, in 1914; the *Encephalartos* was secured from the Botanic Garden at Durban, South Africa, in 1912.

Only the upper scales of the female cone opened, but the pollination seemed efficient, for the cone grew and attained the usual size of cones of *Z. pumila*, instead of degenerating when not pollinated, as do the female cones of all the species of *Zamia* described in this paper. By the middle of October, 1925, the seeds were breaking loose from the sporophylls, and two weeks later the cone fell apart and the seeds were planted (fig. 13). The fleshy layer of the seeds had less of the orange color than the usual seeds of *Z. pumila* and more of the deep red, but not so much as one usually finds in seeds of *Encephalartos villosus*. The seeds have not yet germinated, so that it is doubtful whether any F_1 generation will be obtained. It is well known that pollen of cycads is easy to germinate up to a certain age

in culture solutions. It may be that the pollen germinates in the pollen chamber and stimulates the growth of the cone and ovules, but that no fertilization takes place. One could not remove material for determining such features without danger of killing the entire cone.

All cycads, like most gymnosperms, have twelve and twenty-four chromosomes as the x and $2x$ numbers, which may account in some measure for the ease with which hybrids are obtained in this family.

So far as the names of the plants used in this study are concerned, I cannot speak with authority. For the two species of *Zamia* from Florida we have used the names *Z. pumila* and *Z. floridana*, the names used in WEBBER'S[9] studies of *Zamia* and in various papers and books from this laboratory. The *Z. latifoliolata* came from Porto Rico; the *Ceratozamia mexicana* was grown from seeds secured near Jalapa, Mexico; and the *Encephalartos villosus* came from the Botanic Gardens at Durban, South Africa, and is the form which occurs near Durban.

Records are being kept of the parents and hybrids described in this paper, so that data will be available when the time arrives for an F_2 generation.

I am indebted to P. J. SEDGWICK for the print from which fig. 1 was made, and to C. Y. CHANG for negatives and prints of figs. 2, 5, 8, 9, 10, and 13.

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[9] WEBBER, H. J., Spermatogenesis and fecundation of *Zamia*. U.S. Dept. Agric. Bur. Pl. Ind. Bull. 2. pp. 100. 1901.

CULTURE STUDIES OF PSILOCYBE COPROPHILA

KATHRYN A. GILMORE

(WITH PLATES XXXII, XXXIII)

Psilocybe coprophila was first described by BULLIARD (3) in 1791 as *Agaricus coprophilus*, and was listed under that name by both PERSOON and FRIES. It was placed in the genus *Deconica* by SMITH (12) and subsequent writers who recognize that genus. It is a widely distributed and well known form, occurring most frequently, according to REA (10), on rabbit or cow dung. Culture studies of it were reported by EIDAM (4) in 1875. The cultures used in these experiments all originated from a single basidiocarp that appeared spontaneously on rabbit dung collected in the field near Iowa City, and placed in a moist chamber. The fungus was identified as *Psilocybe coprophila* Fr., in the sense of RICKEN (11), by Professor C. H. KAUFFMAN of the University of Michigan, to whom acknowledgment is herewith extended.

The normal basidiocarp (fig. 1) has a pileus 5–16 mm. broad, at first convex, later becoming expanded and occasionally plane or even depressed. It is glabrous, somewhat hygrophanous, and subviscid when moist; the margin is rather deeply crenate, especially in age; the flesh is very thin; the color ranges from hazel to chestnut brown,[1] fading to warm buff near the margin. The gills are subdistant, unequal, adnate to subdecurrent, warm buff in color, and distinctly mottled at maturity. The stipe is 2–6 cm. in length, 1–2 mm. in the middle, tapering upward, hollow, subcartilaginous, light ochraceous buff, often floccose when young, later the lower half is white tomentose, the upper glabrous and silky-shining. The basidia are tetrasporous, 30–40×10 μ (fig. 15). Flask-shaped sterile cells 20×3–7 μ occur at the edges of the gills (fig. 13). The spores are distinctly purplish in mass when first shed, later blackish brown; cinnamon to snuff brown under the microscope, somewhat apiculate at both ends, and hyaline tipped, 10–12×6–7 μ (fig. 14). They

[1] Names of colors are used throughout this paper in the sense of RIDGWAY.

germinate after about 24 hours in agar, in a liquid nutrient medium, or in water. The formation of a globose vesicle precedes the elongation of the germ tube (fig. 16). Such a vesicle is common in several genera of Basidiomycetes, and was described for the species under consideration by EIDAM.

Vegetative propagation is carried on by means of oidia produced on both the primary and secondary mycelia. These oidia are allantoid, vary from 4 to 10 μ in length, are approximately 1.5 μ in diameter, and are borne on special hyphae. The latter are smaller in diameter than those of normal mycelia, and are branched and coiled and tangled into globose masses 30–40 μ in diameter. They are divided into short segments by cross walls and at length break up, so that each segment becomes an oidium (fig. 8). The oidia appear in single spore cultures not long after the spore germinates. Although they are produced in greater abundance upon the primary strains, they are never lacking upon the secondary mycelia, and have not infrequently been found on hyphae bearing clamp connections (fig. 9). They do not germinate in water, but germinate in 48–60 hours in clear agar.[2] Before germination they commonly become somewhat swollen. A germ tube is first produced at one end; later, the other end usually germinates, so that the oidium is in the center of the mycelium to which it gives rise (fig. 10). The vegetative body of the fungus forms a white cottony mat. Single hyphae of the secondary mycelia normally vary from 1.5 to 2.5 μ in diameter, and when grown in a liquid medium attain a diameter of 3–4.5 μ. They branch freely and possess numerous clamp connections (fig. 11).

The fungus lends itself readily to culture studies because of its large, dark basidiospores, its ability to fruit in culture, and its short life cycle, the whole of which is usually accomplished in less than a month. Test-tube cultures have been made on bean pods, blocks of decayed oak wood, rabbit dung, cow dung, dung agar, malt agar, Cook's II agar,[3] and a modification of the latter containing less nutriment. It is known in this laboratory as IIa agar, and is made according to the following formula:

[2] 15 gm. agar in one liter distilled water; filtered.

[3] COOK, M. T., Del. Agric. Exp. Sta. Bull. 91. p. 11. 1911.

Glucose	10 gm.
Peptone	2 gm.
Dipotassium phosphate	0.25 gm.
Magnesium sulphate	0.25 gm.
Agar	15 gm.
Distilled water	1000 ml.

Basidiocarps occur most consistently on sterilized rabbit dung. Repeated attempts failed to secure their production on horse dung. IIa agar is quite favorable to normal fructification (fig. 2). An effort was made to secure an even better preparation by greater reduction of the nutrients, but without success. Abnormal basidiocarp primordia of various sorts have been produced on Cook's II and on beer agar, but they consistently failed to form true pilei or spores. Mycelial cultures were grown in liquid nutrient media but they did not fruit, even when folds of filter paper were added to provide a foundation for the basidiocarps. Mycelial cultures on both dung and IIa agar where grown in a dark locker, and in a refrigerator where the temperature was 13° C. Fruit bodies with long, slender stipes and abnormally small pilei developed in the dark locker, but mycelial growth was checked by the refrigeration, and no basidiocarps appeared until the cultures were removed to a warmer place, after which growth was renewed and normal fructification occurred. After eight months in culture, April to November, the production of fruit bodies practically ceased during December, January, and February, beginning again early in March.

Heterothallicism

Considerable investigation of the general question of heterothallicism has been carried on in recent years with species belonging to a number of genera of Basidiomycetes. BENSAUDE (1) gives a very complete summary of the work on sexuality in Hymenomycetes up to 1918. She worked with monosporous and polysporous cultures of *Coprinus fimetarius*, and found that a single spore gives rise to a uninucleate (haploid) mycelium; that there are two kinds of spores produced and hence two strains of haploid mycelium; that, if representatives of the two strains are grown in culture together, union will occur between a cell of one and a cell of the other; that a secondary,

diploid mycelium will be initiated as a result of this union, and she believed that fruit bodies are never formed except by a secondary mycelium. She concludes that the fusion between mycelial cells is a true sexual union without the formation of differentiated gametes, and that there is an actual alternation of generations in *Coprinus fimetarius*, the two strains of primary mycelium with a single nucleus in each cell representing the gametophyte generation, and the secondary mycelium resulting from their fusion and bearing two nuclei in each cell representing the sporophyte generation.

KNIEP (**5, 6, 7**) reports studies on sexuality in a number of Basidiomycetes. He agrees with BENSAUDE'S general conclusions concerning the facts on sexuality and alternation of generations in the Basidiomycetes, and is convinced that the vast majority of these forms are heterothallic. In addition he reports the production of fruit bodies by haploid mycelia in *Schizophyllum commune* and *Hygrophorus conica*, and states that in such cases the basidiocarp is haploid throughout; the nucleus of the basidium is not a fusion nucleus and divides without reduction to form the four basidiospore nuclei; the resulting spores are all alike and all produce the same strain of primary mycelium. He finds also, in *Schizophyllum commune* and *Aleurodiscus polygonius*, a situation which he believes to be common in Basidiomycetes, in which four sorts of spores instead of two are produced on each basidiocarp, giving rise to four mycelial strains. Each strain will cross with one only of the other three to produce a secondary mycelium with clamp connections. If primary mycelia from two fruit bodies of *Schizophyllum commune* that grew in different localities are crossed, however, each of the four strains from one basidiocarp will form a secondary mycelium when crossed with any strain from the other. This fact implies that the differentiation of strains depends upon a quantitative balance so delicate as to be destroyed by slight changes in environmental conditions. KNIEP also reports that in two instances primary mycelia which he had held in culture during a year's time, finally changed over into the secondary condition, suggesting again that some quantitative balance within the mycelium had been disturbed by culture conditions.

Further contributions verifying the conclusions of KNIEP and

BENSAUDE have been made by VANDENDRIES (**14, 15**) and MOUNCE (**8, 9**). A concise review of their work, as well as of the unpublished work of HANNA and NEWTON, has been given by BULLER (**2**). The homo or heterothallicism of a number of species has been established, but no reports have been made as yet on the genus *Psilocybe*.

The first monosporous cultures of the species under consideration were made by the poured plate method from the original basidiocarp that occurred spontaneously on rabbit dung. They constitute the F_1 gametophyte generation. Twenty-four such cultures were made, twenty-three of which produced no clamp connections and never fruited, although grown for eight months on various media in twenty-four different transfers. By the end of the eighth month, fourteen of the twenty-three cultures had died and the vigor of the remaining nine was perceptibly reduced. The latter were carried on for two more months and then destroyed. Since no clamp connections were ever found in these monosporous cultures it was concluded that the species is heterothallic, producing at least two sorts of primary mycelium, and that the union of different primary strains is necessary for the formation of a typical secondary mycelium with clamp connections.

The twenty-fourth culture formed clamp connections and fruited regularly under conditions similar to those that brought about fructification in the stock. It is possible that it was in reality not a monosporous but a polysporous culture. It is also possible, however, that it did originate from a single spore, since later a single germinating spore from the stock culture was observed to have given rise to a germ tube in which there was a clearly defined clamp connection at the first septum in close proximity to the spore, under conditions which precluded the possibility of fusion (fig. 12). Such a case was never encountered again in the great number of single spore cultures studied. No further work was carried on with the twenty-fourth culture. Oidia were produced abundantly in it, as well as in the twenty-three haploid cultures.

Numerous crosses of the twenty-three haploid cultures were made to determine the reactions between them. These crosses and the basidiocarps formed by them are referred to as representing the F_1 sporophyte generation. They were examined microscopically for

the presence of clamp connections, and then held in culture to admit of the production of fruit bodies. Not all of the possible crosses were made before some of the single spore cultures died. Enough were made, however, to establish the fact that the spores of *Psilocybe coprophila* give rise to two strains of primary mycelium (known for convenience as strains *m* and *n*), and that clamp connections occur only in those crosses in which both strains are represented. No constant difference between the two strains has ever been found, either in size, appearance, manner of growth, or any other vegetative character. Table I shows the result of the series of crosses involving F_1 gametophytes, a cross indicating the presence, and a dash the absence of clamp connections.

In those cases where members of the same strain were crossed together clamp connections were never produced, and, with one exception, clamp connections always appeared when both strains were represented in the cross. The exception was culture 12–23. Primary mycelium 12 had behaved as a normal representative of strain *m* in eight crosses, and 23 as a normal representative of strain *n* in ten crosses; hence a secondary mycelium was expected as a result of their union. Repeated examinations, however, failed to disclose clamp connections in the cross. The normal basidiocarp that it produced is the only one that has ever occurred on a mycelium without clamp connections. It seemed possible that this basidiocarp was a haploid one, such as has been described by KNIEP (5) in *Schizophyllum commune*, and has since been reported in a number of other genera, in which case it would be expected to produce spores of only one kind. In order to investigate this point, monosporous cultures representing the F_2 gametophyte generation of culture 12–23 were isolated and crossed. Contrary to expectation, clamp connections occurred in many of the crosses, and the monosporous cultures could again be divided into two strains; hence the basidiocarp produced by 12–23 must have borne spores of two kinds, and therefore must have been diploid, in spite of the fact that no clamp connections were found in it. Examination of the mycelium of this culture, made several weeks after the production of the first fruit body, revealed an abundance of clamp connections. It seems improbable that they were present and not seen in the many earlier examina-

tions. Their eventual occurrence may be accounted for by the supposition that spores shed from the basidiocarp had germinated in the culture tube and the resulting mycelia had crossed. No further peculiarities have been noted in the progeny of this cross.

Representatives of the F_2 gametophytes were crossed back with F_1 gametophytes and strains *m* and *n* identified, since one strain of

TABLE I

		Cultures of F_1 gametophytes of strain *n*											Cultures of F_1 gametophytes of strain *m*										
		1	2	4	6	8	10	11	14	15	20	23	3	5	7	9	12	13	16	17	18	19	21	22
Cultures of F_1 gametophytes of strain *m*	22	..	×	..	×	×	×	×	×	..	..	..	..	–	..	..	–	–	–	..	..	..	..	–
	21	×	..	..	..	..	..	×	..	..	..	..	..	–	..	..	..	..	..	..	..	..	–	–	
	19	×	×	..	..	..	..	..	×	×	..	×	..	..	..	–	..	–	–	–	..	–			
	18	..	..	..	..	..	×	×	..	..	..	..	..	..	..	..	..	..	..	..	–				
	17	×	×	..	..	..	..	×	×	..	..	..	..	–	..	..	..	–	–	–					
	16	×	×	..	×	×	×	×	×	..	..	×	..	–	..	–	–	–	–						
	13	×	×	×	..	..	×	×	×	..	..	×	..	..	–	–	..	–							
	12	..	..	..	×	×	×	×	×	..	×	–	..	..	..	..	–								
	9	×	×	..	..	×	..	..	×	..	..	×	..	..	–	–									
	7	..	..	..	..	..	..	..	×	..	..	..	..	..	–										
	5	..	..	..	..	×	..	..	..	..	..	..	–	–											
	3	..	..	..	..	..	..	..	×	..	..	..	–												
Cultures of F_1 gametophytes of strain *n*	23	–	–	..	..	–	–	–	–	..	..	–													
	20	..	..	..	..	..	–	–	..	..	–														
	15	–	..	..	..	–	..	..	..	–															
	14	–	–	–	..	..	–	–	–																
	11	..	–	..	–	..	–	–																	
	10	..	..	..	..	..	–																		
	8	..	..	..	..	–																			
	6	..	..	..	–																				
	4	..	..	–																					
	2	–	–																						
	1	–																							

the F_2 gametophytes formed clamp connections when crossed with representatives of strain *m*, and the other with strain *n*.

Inheritance of abnormal basidiocarps

The great majority of the F_1 sporophytes did not fruit, and many of those which did produced abnormal fruiting bodies. These aberrancies may have been due to certain culture conditions of temperature, moisture, or nutriment; and the fact that similar abnormal forms sometimes occurred in the stock cultures of polysporous origin points to this conclusion (fig. 5). Such stock cultures always pro-

duced normal fruiting bodies on rabbit dung, and the same was true of certain of the crosses. On the other hand, some crosses, notably 11–16 and 10–22, whether on dung or on agar, consistently produced one type of abnormal fruit body having a normal stipe but not a normal pileus. Instead, the apex of the stipe is swollen and hollow, and from the resulting clublike structure there grow out in all directions thin, irregular folds of tissue that function as gills, bearing a hymenium producing viable spores (fig. 3). It seemed possible that the tendency to produce this particular abnormal form might be inherent in the mycelium and transmitted from generation to generation. ZATTLER (**16**) describes such a case in *Schizophyllum commune*, where certain strains carried in their inheritance the tendency to produce "knäuel" fruit bodies, and transmitted it as a simple Mendelian recessive to their offspring. In order to investigate this point, single spore cultures were isolated by the poured plate method from basidiocarps of 11–16 and 10–22.

In the case of 11–16, thirteen monosporous F_2 gametophyte cultures were isolated, five of which were found to belong to one strain and eight to the other. Five crosses that produced clamp connections were held in culture. They have not been very prolific, having produced only six basidiocarps, all on rabbit dung. Of these, one was normal and five were abnormal. None of the abnormal ones was typical of the type described as occurring on the parent stock.

More convincing results were secured with the offspring of cross 10–22. Eight monosporous cultures of the F_2 gametophyte generation were isolated and crossed in every possible combination. Again the presence or absence of clamp connections in the crosses served to divide the monosporous cultures into two strains, and by crossing representatives of each strain with the gametophytes of previous experiments it was possible to identify strains *m* and *n*. Cross 12–23, already described, was used as a check. All of the crosses involving F_2 gametophytes from 10–22 (abnormal) and 12–23 (normal) were made in December 1924, and held in culture on rabbit dung during the same period of time and under the same conditions. The record of basidiocarp formation in the two cases affords an excellent opportunity for comparison of the fructification of the offspring from a cross producing a preponderance of normal with one

producing a preponderance of abnormal basidiocarps. Sixteen crosses were made that involved F_2 gametophytes from the normal culture 12–23, forming fifteen basidiocarps. Thirteen of these were entirely normal. Two, both produced as the result of a single cross, were merely convolute masses of hymenial tissue without a stipe, described later as the third type produced by the abnormal strain. Seventy-three crosses were made involving F_2 gametophytes from the abnormal culture 10–22, producing ninety-six basidiocarps. These could be divided into four types, not sharply distinguished, but grading into one another. The first type is the normal basidiocarp (fig. 1). Thirty-seven of these were produced, sixteen of which occurred in crosses between F_2 abnormal gametophytes and F_1 or F_2 normal gametophytes. The second type has a stipe and an abnormal pileus. Representatives of this type vary widely in the shape of the pileus and in the distribution of the hymenium-bearing tissue (fig. 7); many of them approach the typical abnormal form already described. Seventeen basidiocarps of this type were produced. The third type has neither stipe nor pileus; instead, a mass of basidiocarp tissue of no regular shape is formed. It may grow horizontally over the substratum, or it may become erect; but at its apex it always produces more or less hymenium-bearing tissue in folds and convolutions of various sorts. Nineteen basidiocarps of this type were produced. These three types all produce viable spores. The fourth type is simply a mass of basidiocarp tissue and produces no spores (fig. 6). Twenty-three such abortive bodies were produced; hence the crosses involving F_2 gametophytes of 10–22 (abnormal) produced normal and abnormal basidiocarps in the ratio of 1–1.6, while the crosses involving F_2 gametophytes of 12–23 (normal) produced normal and abnormal basidiocarps in the ratio of 6.5:1. Since the two series fruited during the same interval of time and under the same conditions, it is obvious that the capacity to form normal or abnormal fruit bodies is affected by inheritance. It cannot, however, be expressed in a simple Mendelian ratio. Many F_2 sporophytes from the abnormal cross produced both normal and abnormal basidiocarps from the same mycelium, a circumstance that would not occur if the capacity for forming abnormal basidiocarps were either strictly dominant or strictly recessive to the capacity for forming

normal ones. It is possible that the tendency to fruit abnormally is present in the mycelium of these crosses, but is able to express itself only under certain environmental conditions. The amount of moisture present is possibly the critical factor, since it has been the most variable one and the hardest to control.

It seemed desirable to make some study of the F_3 generation of an abnormal strain, and accordingly single spore cultures were isolated from a basidiocarp formed by sporophyte 46–48. Cultures 46 and 48 were F_2 gametophytes from the abnormal strain 10–22, and the secondary mycelium produced by crossing them had formed both normal and abnormal basidiocarps. Single spore cultures isolated from one of the normal basidiocarps were crossed with each other and with F_2 gametophytes from the normal cross 12–23. These crosses produced nine basidiocarps, two abnormal and seven normal. Fig. 4 shows a normal and an abnormal basidiocarp produced simultaneously in one of these cultures.

Oidial studies

The oidia of this species were described by EIDAM (4). Working long before the sexuality of the Basidiomycetes was understood, he believed the oidia to be male cells or spermatia. He looked in vain for the female organs, and concluded that they appeared less frequently than the spermatia, which were common and abundant so as to insure fertilization. Oidia similar in appearance and in manner of formation have been found repeatedly on the primary mycelia of other Hymenomycetes. VANDENDRIES (14) reported their occurrence on *Hypholoma fasciculare*, and THOM and LATHROP (13) found them on the secondary mycelium of a form which they isolated from fermenting bagasse and believe to be *Psilocybe atomatoides* Pk.

It seemed worth while to compare the reactions of oidia occurring upon the primary and secondary mycelium of *Psilocybe coprophila*. The poured plate method was used to isolate cultures arising from single oidia. The oidia are hyaline, and so small that it is difficult to be sure that a given field in the agar plate contains but one; consequently the possibility of error is greater in all of the oidial experiments than in those depending upon the isolation of the large, dark basidiospores.

Six single oidial cultures were isolated from F_1 gametophyte 13 of strain *m*, and six from F_1 gametophyte 14 of strain *n*. Clamp connections were not found in any of these cultures. In crosses they behaved exactly like the parent cultures from which they were derived; hence it was concluded that the oidia from a haploid mycelium of either strain are haploid, as would be expected, and give rise to haploid mycelia of the same strain.

Eleven single oidial cultures were isolated from the stock culture of polysporous origin. Each of them produced a diploid mycelium

TABLE II

	02*	05	07	01	03	04	06
54	×	×	×	−	−	−	−
26	×	×	−	×	×	×	×
06	−	−	×	−	....	....	−
04	....	....	....	....	....	−	
03	....	....	....	....	−		
01	−	−	×	−			
07	×	×	−				
05	−	−					
02	−						

* Cultures 02 and 05 are single oidial cultures from the F_1 sporophyte 14–19, which formed a secondary mycelium when crossed with representatives of either strains *m* or *n*.

Culture 07 is a single oidial culture of strain *n* from the F_2 sporophyte 48–46.

Cultures 01, 03, 04, 06 are single oidial cultures of strain *m* from the F_2 sporophyte 14–19.

Culture 26 is an F_2 gametophyte of strain *n* produced by F_1 sporophyte 12–23.

Culture 54 is an F_2 gametophyte of strain *m* produced by F_1 sporophyte 12–23.

with clamp connections. Two weeks later two additional cultures of the same sort were isolated from another stock culture of polysporous origin, and these also were diploid.

Six single oidial cultures were isolated from the F_1 sporophyte 14–19 and one from the F_2 sporophyte 45–48. Unlike the oidial cultures from stock, these seven were all haploid. Crosses involving them were made, and are recorded in table II. Culture 54 is a representative of strain *m*, and 26 of strain *n*. No explanation is offered for the fact that cultures 02 and 05 formed clamp connections when crossed with either 54 or 26; nor for the fact that, having crossed with both 54 and 26, they refused to cross with each other, or with cultures

01 and 06. Bacterial contamination that was present in this series of crosses may have interfered with normal reactions. The five cultures other than 02 and 05 reacted as normal haploid mycelia; 01, 03, 04, and 06 representing strain *m*, and 07 representing strain *n*.

The suggestion presented itself that the oidia which gave rise to these cultures were perhaps not borne on the secondary mycelia of the crosses 14–19 and 45–48 from which they were taken, but on the primary mycelia 14, 19, 45, and 48, which might still be maintained in the culture tubes. In order to investigate this possibility, transfers of mycelium from cross 14–19 were made to agar plates. While the resulting cultures were still young, the tips of radiating hyphae with clamp connections, but not bearing oidia, were cut off and transferred again. Thus plate cultures were prepared which had their origin from secondary mycelium only, and could not be mixed cultures bearing both diploid and haploid hyphae and oidia. This experiment was repeated several times, and in every case, after a few days, oidia appeared abundantly in the culture. An attempt was made to isolate cultures from single oidia produced by such cultures, but only two of them lived. These two were both haploid.

Eight single oidial cultures were isolated from the F_2 sporophyte 52–53. Seven of them were haploid and the eighth diploid. Since the oidia from stock and those from known crosses had behaved differently, in spite of the fact that both came from diploid mycelia, it seemed wise to check the results obtained from the stock oidial cultures. Four oidia from stock were isolated six weeks after the first ones had been secured. This time all four produced not diploid, but haploid mycelia without clamp connections.

The behavior of oidia from diploid cultures has been so variable that interpretation is difficult. Seventeen single oidial cultures have been isolated from diploid mycelia formed by crossing two primary strains. Oidia from three such crosses were used. Of these, sixteen were haploid. Two of these haploid cultures originated from oidia that were produced on plates where only a diploid mycelium was growing, so they could not have been formed by the original haploid strains that united to produce the diploid mycelium; hence diploid mycelia must give rise, at least in some cases, to haploid oidia. In order that this may take place there must be a segregation of nuclei

within the secondary mycelia. Either portions of growing secondary mycelia are continually breaking down into the primary condition and these portions produce the oidia, or segregation of nuclei occurs in diploid mycelia at the time of the formation of oidia, making the oidia haploid while the mycelia that bear them remain diploid.

The seventeenth oidial culture from a known cross was diploid. It might have originated from a small bit of diploid mycelium or from two unlike haploid oidia erroneously taken for a single germinating oidium. On the other hand, the belief that it originated from a single diploid oidium is supported by the fact that so many diploid cultures were secured from oidia produced by stock cultures of polysporous origin. In the latter case, the first thirteen cultures were all diploid, and it seems extremely unlikely that so many errors were made in isolating cultures; hence, diploid mycelia must sometimes give rise to diploid oidia. Later oidial cultures from an older stock culture were all haploid. This implies again that segregation of nuclei had taken place. But since the ratio of diploid to haploid cultures from stock oidia is 13:4, and only 1:16 in the case of oidial cultures from a known cross, and since the stock cultures from which the thirteen diploid oidial cultures were isolated were younger than those from which the four haploid oidial cultures were isolated, it is evident that segregation must take place less readily in cultures of polysporous origin.

Summary

1. *Psilocybe coprophila* Fr. has been found to be a heterothallic species producing two strains of primary mycelium, the union of which is necessary for the production of a secondary mycelium with clamp connections, and of basidiocarps.

2. The capacity for the formation of abnormal basidiocarps is greater in some crosses than in others, and is transmitted from generation to generation, although not as a simple Mendelian character.

3. Oidia are produced on both primary and secondary mycelia. Those produced by primary mycelia germinate to form haploid mycelia of the same strain as the parent culture. Those produced by secondary mycelia sometimes germinate to form diploid mycelia. At other times they germinate to form haploid mycelia, segregation

of nuclei occurring before the oidia are produced. This segregation seems to take place less readily in cultures of polysporous origin than in a diploid mycelium formed by crossing two primary strains.

This work was done in the botanical laboratory of the University of Iowa, under the direction of Professor G. W. MARTIN, for whose assistance the writer is much indebted.

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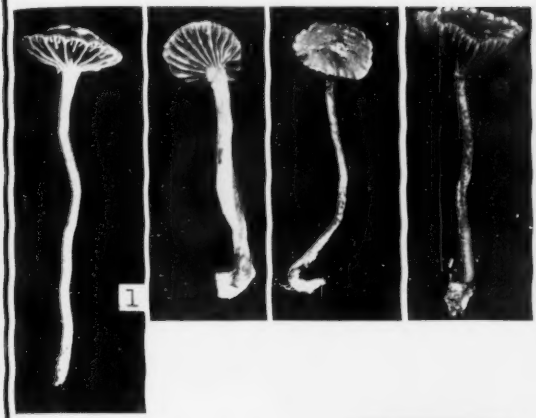
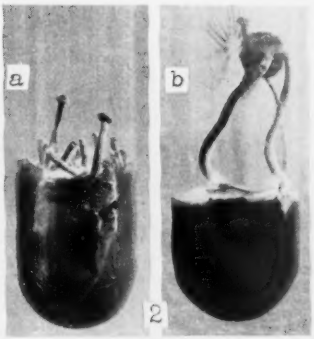
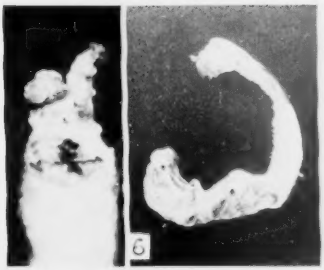
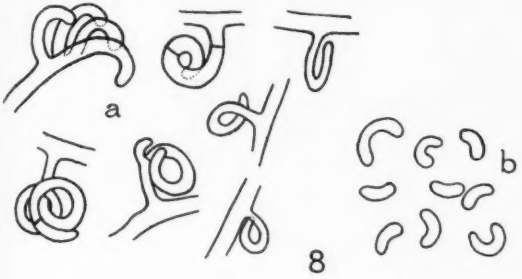
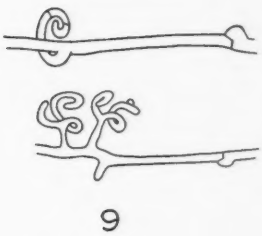


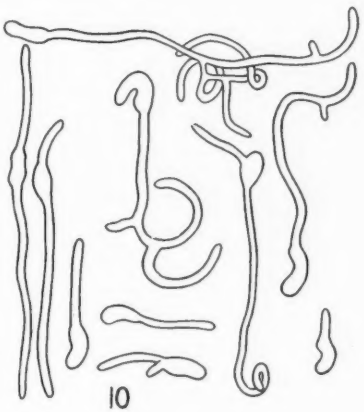


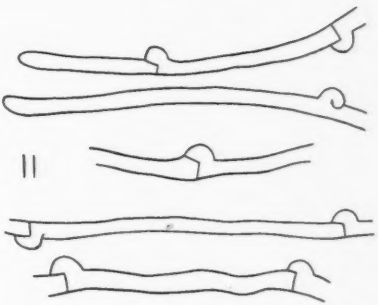


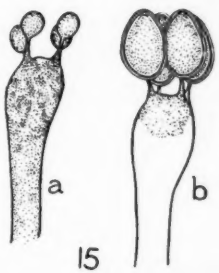
GILMORE on PSILOCYBE

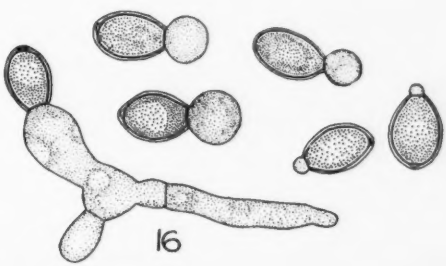












GILMORE on PSILOCYBE

EXPLANATION OF PLATES XXXII, XXXIII

PLATE XXXII

FIGS. 1–7 photographed natural size; reduced one-eighth in reproduction.

FIG. 1.—Normal basidiocarps of *Psilocybe coprophila*.

FIG. 2.—Normal fructification of stock culture on IIa agar: *a*, primordia; *b*, mature basidiocarp.

FIG. 3.—*a*, Abnormal basidiocarp of type produced by cultures 11–16 and 10–22: *b*, same basidiocarp cut longitudinally.

FIG. 4.—Normal and abnormal basidiocarps produced simultaneously by F_3 sporophyte culture on rabbit dung.

FIG. 5.—Abnormal basidiocarps produced by stock culture on IIa agar.

FIG. 6.—Masses of undifferentiated basidiocarp tissue.

FIG. 7.—Various types of abnormal basidiocarps with stipes and pilei.

PLATE XXXIII

FIGS. 8–16 drawn with camera lucida; figs. 8, 11, 13–16×1920; figs. 9, 10, 12×1515; reduced one-half by reproduction.

FIG. 8.—*a*, oidiophores; *b*, oidia.

FIG. 9.—Oidiophores occurring in close proximity to clamp connections.

FIG. 10.—Germinating oidia.

FIG. 11.—Clamp connections from stock cultures.

FIG. 12.—Germinating spore with clamp connection at first septum of germ tube.

FIG. 13.—Flask-shaped sterile cells occurring at gill edges.

FIG. 14.—Basidiospores.

FIG. 15.—Basidia: *a*, young; *b*, mature.

FIG. 16.—Germinating basidiospores.

OSMOTIC PRESSURE OF CELL SAP AND ITS POSSIBLE RELATION TO WINTER KILLING AND LEAF FALL

FLOYD W. GAIL

(WITH THREE FIGURES)

Introduction

The fact that most coniferous trees and some of the angiosperms do not shed their leaves every year has attracted considerable attention among scientists interested in plant life. The large number of evergreen plants in Idaho, Oregon, and Washington was instrumental in causing the writer to begin this investigation. Numerous studies have been made on the density and sugar content of the cell sap, but, so far as the writer has been able to ascertain, no studies have been made at regular times throughout an extended period. The work here reported covered nearly three years.

SCHULTZ (**15**) studied reserve materials in evergreen leaves in winter. All gymnosperms studied were found to be free from starch except *Gnetum gnemon*, in which the green cells contained some starch granules. Among the angiosperms, all monocotyledons and some dicotyledons were free from starch. A few contained more or less starch in the leaves. According to the investigations of MER (**10**), LIDFORSS (9), and others, almost all of the evergreen plants of northern and middle Europe lose the starch from the green cells of the leaves during winter. They have also shown that a close relation exists between the sugar content and frost hardiness. MIYAKE (**11**) found that, generally speaking, starch in evergreen leaves began to decrease in November, reaching its minimum during January and the beginning of February, and increased again from the end of February. Many evergreen leaves in Tokyo and other parts of middle and southern Japan contain more or less starch, while it is entirely absent in some species in the coldest time of winter. The majority of evergreen leaves in the northern part of Japan lose nearly all of their starch from the mesophyll and guard cells in winter.

WARMING (17) considers that the presence of oils and fats in leaves in the winter may enable the plant to withstand low temperatures, in that fatty oil in the form of an emulsoid prevents the subcooling of the plant tissue and increases the power of resistance to frost. TUTTLE (16) worked on the reserve material in *Linnaea*, *Pyrola*, and *Picea*, and found that most evergreen plants are destarched in northwestern Canada as early as October, and then contain a large amount of oil. Exposure to high temperatures in the case of *Linnaea* induces the formation of starch in darkness. The starch disappears again when exposed to moderately low temperature for about eight days, but the leaves are killed if exposed to extremely low temperatures when filled with starch. LEWIS and TUTTLE (8) have shown that the maximum osmotic pressure is reached in *Picea* and *Linnaea* toward the end of March. *Pyrola* shows a fairly steady decrease from the middle of December until June. The variation of the sugar content closely follows the variation of the osmotic pressure. The sugars show a decided concentration during the winter months. In the living leaves of *Pyrola*, ice formation does not begin until a temperature of $-31.6°$ C. is attained.

HARVEY (4) found that the relative injury to varieties of cabbage and lettuce at low temperatures is dependent upon varietal differences in the ability of these plants to harden. Cabbage which had been placed in dark chambers and exposed for five days to 3° C. was not injured by thirty minutes, exposure to $-3°$ C., although frozen stiff. He also found the average excess of the freezing point depression in the cell sap of hardened cabbage plants over the cell sap of non-hardened to be nearly 0.1°. He considered this slight difference insufficient to account for the resistance to low temperature shown by hardened plants which are not injured by being frozen at a temperature 3° below the killing temperature for non-hardened plants.

MÜLLER-THURGAU (12) in 1880 gave the freezing point of potatoes as $-1.8°$ C. WRIGHT and HARVEY (18) report that the freezing point of potatoes tends to rise as the season advances. HARVEY and WRIGHT (6) state that, during the usual weather conditions which precede the first killing frost in autumn, the night temperature is usually somewhat above 32° F., but low enough to increase

the accumulation of osmotically active sugar in tomatoes with a consequent lowering of the freezing point of the plant sap. WRIGHT and TAYLOR (19) state in their summary that potatoes freeze more quickly when exposed to rapidly diminishing temperature than when it falls slowly.

KORSTIAN (7) found that the density of the cell sap changes during the day, having a minimum in the morning, increasing as the day advances, and decreasing toward evening. Newly developed leaves have uniformly lower sap densities in July than one-year-old leaves. In September the relative densities were found to be variable; about one-half of the species showed higher density in the leaves of the current year, and the other half in those of the second year. By December and January, however, all of the species tested showed uniformly higher densities in the second year leaves. Starch was not detected in any of the conifers or evergreen shrubs in either January or February. Osmotic pressure in plants is more rapidly changed by fluctuation in moisture conditions of the site than by temperature or light.

Method

It is well known that the osmotic pressure is proportional to the freezing point depression. In this investigation, the freezing point depression was determined by the use of the Beckman thermometer, and the osmotic pressure was computed on the basis of the freezing point depression.

The needles from the current year's growth only were picked from the trees. They were then at once ground through a meat grinder, using the fine knife. In most cases this pulp was frozen and then the sap pressed out, using a definite amount of pressure and a definite amount of pulp. Little difference in the freezing point depression resulted from freezing the pulp. The fact that there was so little difference in the freezing point depression produced by freezing the pulp probably may be explained by the fact that the cells of the leaves were so largely broken by being ground into the pulp. The freezing point depression was then determined. Tests were made at least once a month for a period of nearly three years.

The non-deciduous trees used retained their needles four or more years. The non-deciduous trees used were *Pinus ponderosa*,

Pseudotsuga taxifolia, and *Abies grandis*. The non-deciduous, broad leaved shrubs used were *Ceanothus velutinus* and *Pachistima myrsinites*.

Discussion

Non-deciduous trees and shrubs

The cell sap was tested each month throughout the years 1922 to 1924 inclusive. Fig. 1 shows that the osmotic pressure of the cell sap in every species studied increased very rapidly during December

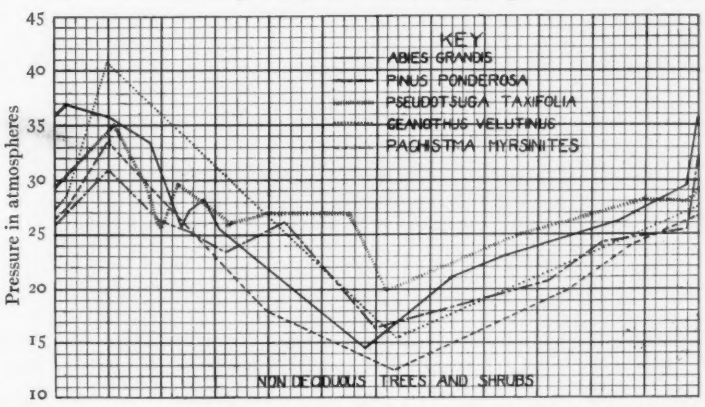


Fig. 1

and January, and that the highest osmotic pressure was during the last week of January or the first week of February. The osmotic pressure then decreased until the last week of June or the first two weeks of July. This indicates that the growth in this region ceases largely by the middle of July. Photosynthesis, however, continues but the products are stored. This causes a gradual increase in osmotic pressure in all species during August, September, October, and November (fig. 2).

Lewis and Tuttle likewise found that the osmotic pressure of the cell sap of *Pyrola* showed a fairly steady decrease from the middle of December until June. Korstian also reports a similar fact, but attributes the increased osmotic pressure to the dry season.

This he considers reduces the amount of available water, and as a result the osmotic pressure is increased. In this region the dry season begins about July, continuing for about three months. During this time the osmotic pressure does commence to increase gradually. The rainy season usually begins in October or November, however, but the osmotic pressure still continues to increase. This leads the writer to believe that there is some other factor than the reduced amount of available water which causes increased osmotic pressure.

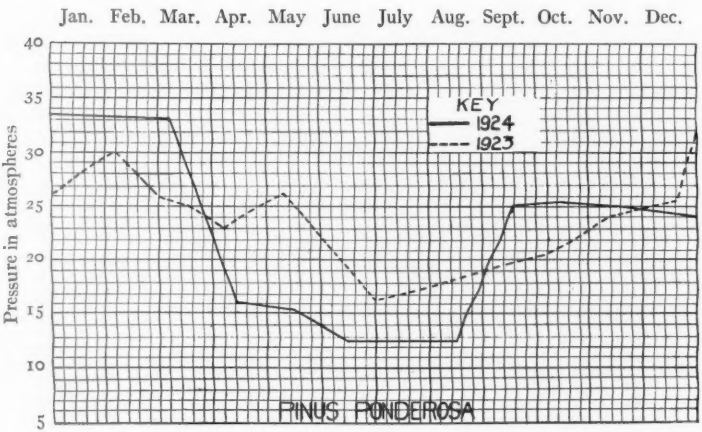


FIG. 2

Perhaps the gradually increasing osmotic pressure during August, September, and October is due largely to the cessation of growth and accumulation of carbohydrates not used in growth. The growth may have been inhibited by the dry season. The continued increased osmotic pressure of the cell sap during November and December, when there was an abundance of water in the soil but a lower temperature and little or no growth, the writer attributed to the lower temperature. The enzymes at this time are changing some of the starches to sugars, oils, etc., which increases the osmotic pressure.

The highest osmotic pressure recorded was 40.08 atmospheres in *Ceanothus velutinus*. This was during the last week of January.

The lowest osmotic pressure was recorded as 10.51, during the second week of July. This was in the cell sap of *Pachistima myrsinites*. Repeated readings demonstrated that, as the altitude increased and the temperature was lower, there was increased osmotic pressure in the same species. The fluctuations in the curves (fig. 1) showing the osmotic pressures during February and March are the results of cloudy days alternating with days of sunshine. Continuous cloudy weather caused a lowering of the osmotic pressure, since the amount of carbohydrates produced by photosynthesis is reduced, and at the same time growth is probably taking place.

The osmotic pressures shown in fig. 1 are the averages for the two years 1922 and 1923. The investigation was continued, however, to March 1925. Fig. 2 shows the average osmotic pressure for *Pinus ponderosa* for the two years (taken from fig. 1), and also for the year 1924. The average osmotic pressures of the cell sap for the two years is quite comparable with the osmotic pressure of the cell sap for the year 1924 until the month of December. The average osmotic pressure for the years 1922 and 1923 increased rapidly during the last two weeks of December, while for the year 1924 it did not increase during December or through the remaining winter months. Table I shows the temperatures for the month of December for the years 1923 and 1924, as recorded by the United States Department of Agriculture, Weather Bureau of Latah County of the State of Idaho. It will be seen that, in December 1923, the temperature varied little until the thirtieth, when the temperature was 20° F. and on the thirty-first it was −2°. This was followed by four days in January of zero weather or below, but the lowest temperature was −7°. The greatest drop in a single day was 22°. This was followed by temperatures considerably above zero. The highest temperature during the entire month was 45°. During 1924 the highest temperature for the same month was 56°, and it will be seen (table I) that in twenty-four hours there was a drop of 60°. This was followed by nine days when the temperature went as low as −17° on two days, and at no time was it above zero. The temperatures in the Selway National Forest were similar to those for Latah County, but the extremes were even more severe. A drop of 67° took place in less than forty-eight hours. This was followed by eleven days when the

temperature was −19° for two days and below zero every day except one, when the temperature was 0° F.

It is an interesting fact that, during the years 1922 and 1923, the needles of the *Pinus ponderosa* forests did not winter kill. During the winter months of 1924–1925, however, they were completely winter killed in large areas. This was true in the Spokane region, in the Nez Perce prairies, to a considerable extent in Latah County, and in other regions of northern Idaho, eastern Washington, and

TABLE I

TEMPERATURES (°F)

YEAR	DECEMBER																			
	1	7	14	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1923.....	28	36	36	45	45	45	36	37	36	34	26	35	30	33	31	43	31	20	− 2
1924.....	45	30	56	− 4	−17	−17	−10	− 2	0	−14	−12	− 4	4	9	17	21	38	39	39

parts of Montana. Most of the trees were not killed, since they developed new needles from the buds. Since the killing of the needles did not occur in either 1922 or 1923, when there was no such sudden drop of temperature, the writer is inclined to attribute it to the sudden lowering of the temperature which occurred in December 1924.

LEWIS and TUTTLE found that the variation of the sugar content closely follows the variation of osmotic pressure. The sugars show a decided concentration in winter in the case of *Pyrola*.

PEARSON (**13**) states that when the soil temperature falls to 32° F. or even a few degrees above 32°, the soil moisture ceases to be available. If this condition persists continually over a long period during which transpiration is favored by sunshine and wind, the result may be fatal to a tree which is unable to endure extreme desiccation. The temperature up to the time of the sudden drop was unusually high, being 40° or higher most of the time.

The ground at the time of the sudden drop in temperature was practically destitute of snow. PEARSON states that, during the snowless period, the soil temperatures taken in the open down to six inches in depth are usually several degrees higher than the mean or even the maximum air temperatures. Since the period of low tempera-

tures was nine days and the temperature of the air preceding this time was unusually high, the temperature of the soil, for any considerable length of time, could not have been below the point (32° F.) at which the soil moisture ceases to be available.

During the winter of 1917, GAIL (**2**) found that there was winter killing of the needles of *Pinus ponderosa*. The wind blew continuously and very rapidly from the southwest for a considerable period of time. The following spring the needles were found to be brown and killed on the exposed side of the trees, while on the protected side they were alive and green. The average velocity of the wind per hour during the cold spells of January 1924 and of December 1924 were respectively 11.4 and 11.53 miles. The needles of the yellow pine were not killed in any of the regions mentioned during the cold spell of January 1924, but they were killed the next winter during the cold spell of December 1924. The difference in the velocities of the wind was only 0.13 of a mile per hour. The cold spell of December 1924 did continue approximately four days longer than the cold spell of January 1924. These two differences, being so little, can hardly explain the winter killing of the needles of the later date. The wind velocity was not excessive in either January or December of 1924. The prevailing wind was from the southwest, but all of the needles on most of the trees were killed in December 1924. Had this been due to wind, the leaves on the southwest side only would have been killed, since the prevailing winds were from that direction. The writer does not consider the winter killing of the needles was due to desiccation brought about by either the low temperature of the soil or evaporation due to the wind. The low temperature in itself was not the cause, but rather the sudden fall of the temperature. This was so sudden that the starches were not changed to sugars, oils, etc., which resulted in the osmotic pressure of the cell sap not being increased, as fig. 2 shows, and consequently the protoplasm of the cells was frozen. This the writer considers resulted in the winter killing of the needles. This seems to be in accord with the work of TUTTLE, who found the leaves of *Linnaea* were killed if exposed to extremely low temperature when filled with starch. It also agrees with the work of WRIGHT and TAYLOR on potato freezing.

DECIDUOUS TREES AND SHRUBS

The trees used were *Larix occidentalis* and *Fraxinus lanceolata*. Tests to show the osmotic pressure of the cell sap were begun as soon as the leaves were sufficiently well developed, which was about the middle of May. Tests were continued once each month or more until the falling of the leaves in early November. Fig. 3 shows the osmotic pressure in May to be about 15 atmospheres for both trees.

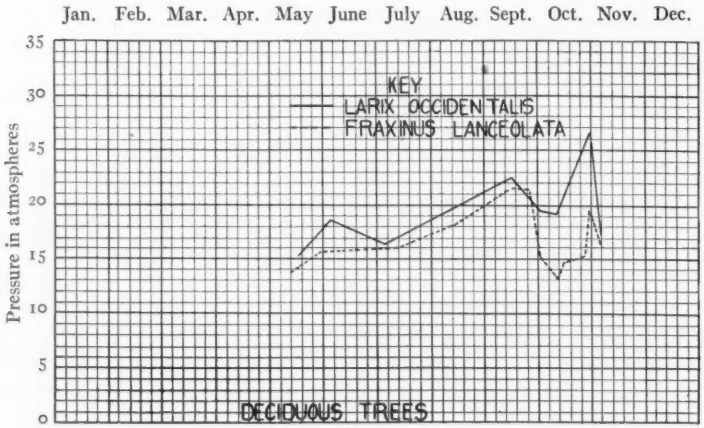


FIG. 3

This increased for both trees slightly until about the middle of September, when there was a decrease. About the middle of October the osmotic pressure increased again rather rapidly. It increased to 26.53 atmospheres in *Larix occidentalis* and to about 19.5 atmospheres in *Fraxinus lanceolata*. The temperature became lower the last week of October; heavy frost occurred during the first week of November, and the leaves were shed November 5. The osmotic pressure of the leaves at this time (immediately after being shed) had dropped to 8.43 atmospheres in *Larix*, and to 5.4 atmospheres in *Fraxinus*. This would seem to indicate that there had been a transfer of the sugars from the leaves to the trunk, and that as a result the osmotic pressure decreased. Tests were made on alternate days during the last week of October and the first week of November, in

order to determine as accurately as possible the changes that were taking place.

Conclusions

1. The lowest osmotic pressure in the cell sap of the non-deciduous plants studied is during the last half of June and the first half of July.

2. There is a gradual increase in the osmotic pressure of cell sap in the non-deciduous plants studied, throughout the months of August, September, October, and November. The stored starches are changed to sugars, oils, etc., which increase the osmotic pressure. The change of starches to sugars and oils is brought about by enzymes as the temperature lowers.

3. Growth of the conifers under consideration practically ceases by the middle of July. The drought during July, August, and September may inhibit growth. Photosynthesis continues, however, and the products are stored, resulting in increased osmotic pressure.

4. There is usually, in the non-deciduous plants studied, a sudden increase in the osmotic pressure of the cell sap during December and January. If the temperature drops low enough too suddenly, however, the osmotic pressure does not increase. This the writer considers results in the freezing of the protoplasm, and, if the freezing is severe enough, kills the leaves, causing them to turn brown and fall. It may even kill the entire plant.

5. That the osmotic pressure does not increase is probably explained by considering that the sudden drop in temperature does not give sufficient time for the enzymes to change the starches to sugars, oils, etc., before freezing takes place. This perhaps explains the winter killing of the needles of *Pinus ponderosa* which took place in December 1924.

6. Reduced amount of light, due to cloudy weather, in both non-deciduous and deciduous plants studied, causes a decrease in the osmotic pressure of the cell sap.

7. There is a consistent increase in the osmotic pressure of the cell sap of the non-deciduous trees and shrubs studied as the temperature becomes lower during the fall and winter months.

8. There is no consistent increase in the osmotic pressure of the

cell sap of the deciduous trees studied, as the temperature becomes lower in September, October, and November.

9. The osmotic pressure increases with the same species of non-deciduous plants studied, growing in different altitudes as the altitude increases.

Acknowledgments are due to Mr. E. M. KEYSER, assistant meteorologist, United States Weather Bureau, Spokane, Washington, for wind data; to Mr. M. L. MARSH of the United States Forest Service, Kooskia, Idaho, for temperatures in the Selway National Forest; and to Mr. H. A. BRUENN, Winchester, Idaho, for other temperature data.

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STRUCTURE AND CULTURAL HISTORY OF A MYCETOZOAN FOUND IN TOBACCO PLANTS WITH MOSAIC-LIKE SYMPTOMS[1]

(WITH PLATES XXXIV–XXXVII AND TWO FIGURES)

PHILIP M. JONES

Introduction

The purpose of this paper is to give a description and the life history of a mycetozoan obtained from tobacco plants affected with mosaic-like symptoms. The organism is provisionally placed in the genus *Plasmodiophora*, because it is nearer the structure of this genus than any other which has been described. It should be noted, however, that its method of depositing spores is different from any *Plasmodiophora* hitherto described. As the species is probably new it is named **P. tabaci,** n. sp.[2]

Methods

To demonstrate the presence of the organism in tobacco tissues the following method is used. The tissue is killed with osmic acid vapor and then fixed in Schaudinn's fluid without acetic acid for one hour. Following this, the tissues are dehydrated and imbedded in paraffin, sectioned, and stained in Haidenhain's haematoxylin (long method). Even better results are obtained when the tissues are placed in Knop's solution for three days before fixation.

The organism is cultured from tobacco tissues in a dilute Knop's solution. One gram each of KNO_3, KH_2PO_4, Mg_2SO_4, and $Ca(NO_3)_2$, and a trace of ferric phosphate are dissolved in 200 cc. of distilled water. This solution is placed in Petri dishes or flasks with cover glasses, and then sterilized at 15 pounds for one half-hour. The plant tissues are cut into small pieces, washed in 1–1000 mercuric chloride solution, rinsed repeatedly with sterile water, and then placed in the

[1] This paper is being published on its recent receipt, at the expense of the author.

[2] JONES, P. M., A mycetozoan found in tobacco plants with mosaic-like symptoms. Abst. in Phytopath. **16**:67. 1926.

Knop's solution and allowed to incubate 7–15 days. By this method either amoebae or flagellates or both are obtained. By repeated transfers the organism was maintained in culture for over two years. To study the organism the cover glasses are removed, wiped on the under side, and examined under the microscope.

For cytological studies the organism is killed by exposure to osmic acid fumes for one-fourth minute, and then placed in Schaudinn's solution (95 per cent alcohol saturated with mercuric chloride). The fixed material is stained in Haidenhain's iron haematoxylin (short method), and mounted in balsam.

Occurrence in tissues of tobacco plants

By the method just given, plasmodia have been demonstrated in the tissues of the tobacco plant. The organism is intracellular, flowing from cell to cell by its pseudopodia (figs. 1, 3, 4, 59). It may lie close to the cell wall, or around the nucleus or the chloroplasts. Little dots frequently are seen on the periphery of the nuclei and of the chloroplastids. These dots seem to be similar to the inclusions found in the cysts which the organism forms when cultured in Knop's solution. Often the nucleus of an infected cell is found in division. Disintegration of chloroplasts apparently is the first visible response on the part of the cell to the presence of the organism. Later the cell walls disintegrate and disappear (fig. 4).

Life cycle in culture

By changing the food, temperature, and moisture conditions of the culture medium, the various stages of the life cycle have been determined. The amoebae and flagellates which develop when the tobacco tissues are placed in Knop's solution become encysted under proper conditions. Each of these cysts later develops into an amoeboid organism. A number of these may fuse to form a plasmodium, which in turn gives rise to free spores, in a way characteristic of this species. These free spores, after encysting, give rise to amoebae of the "limax" type.

In the cyst stage of the life cycle of the mycetozoan in culture there occur inclusions which may be parasites of the *Plasmodiophora*. So far, the plasmodium, with or without bodies which appear similar

to the inclusions found in the cysts in culture, is the only stage which has been found in killed and stained tobacco tissues. Amoebae and flagellates which differ somewhat in shape and size from those

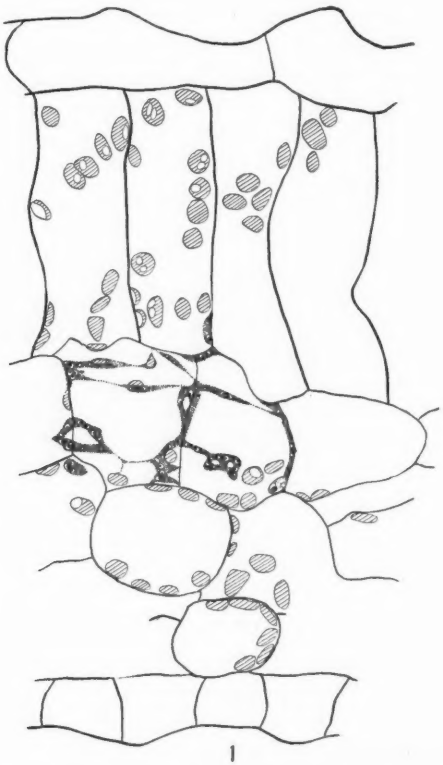

FIG. 1.—Semi-diagrammatic camera lucida sketch of section through leaf of tobacco with mosaic-like symptoms, showing plasmodium in mesophyll; ×760.

obtained from tobacco tissues, have been cultured in Knop's solution from tomato plants affected with mosaic, from potato plants affected with leaf roll, and from the intestines of aphids feeding on tobacco and tomato plants with mosaic-like symptoms and on potato plants showing the symptoms of leaf roll (figs. 71–75).

Amoeboid stage

Morphology of "limax" type.—The endoplasm is filled with food vacuoles which contain bacteria in process of digestion. These bacteria probably are carried into the culture by the plant tissues. Some amoebae were found to be full of food vacuoles, especially at the posterior end; some had none; and in the majority of cases there were a few only (figs. 5, 6, 7). The contractile vacuole is variable, being present in some cases and absent in others. When present it is always posterior to the nucleus.

The resting nucleus is spherical in shape with a distinct nuclear membrane (fig. 12). Between this and the small karyosome lies a clear area. The karyosome is centrally located and appears as a large compact sphere which sometimes stains homogeneously; in other cases the central portion stains much less darkly than the periphery, and a small deeply staining granule, which may be a centriole, can be seen in the center.

Movement.—The body of the amoeba has one constant character, namely, it progresses by means of one blunt broadly rounded or lobose anterior pseudopodium (figs. 12, 68). While the general direction of movement is in a straight line, there is not a constant flowing movement, but rather an alternation of pseudopodium formation from side to side (fig. 14). When not in locomotion an amoeba often alternately draws in and thrusts out a pseudopodium on either side, advancing the one while withdrawing the other. This may take place at any point and may be repeated indefinitely. The floating forms have 3–6 short pseudopodia (fig. 13). In the flowing forms the cytoplasm is clearly differentiated into ectosarc and endosarc, the latter being conspicuously thick at the anterior end (figs. 12, 14). In floating forms no such differentiation was observed.

Binary fission.—During division the amoeba does not necessarily round up, although all movement of the organism ceases (fig. 16). The division of the nucleus is promitotic, that is, large chromatic polar masses are formed within the nuclear membrane and fibrils appear between them. The beginning of nuclear division is characterized by an increase in nuclear size (fig. 17). The nuclear membrane becomes more distinct and the peripheral chromatin more evident than in the resting nucleus. The central karyosome then

increases in size, elongates, and assumes a bent dumb-bell shape (fig. 18). The peripheral chromatin at the same time migrates in the nuclear membrane toward the karyosome. Spindle fibers then appear between the chromatic polar masses, which then move apart (figs. 19–22). The nuclear membrane constricts in the middle and the two daughter nuclei separate. The polar mass then assumes the shape of the karyosome, and the resting nucleus is reconstructed. Although I have searched very carefully for the chromosomes during the process of division, I have not been able to differentiate them from the spindle fibers. At the same time that reconstruction and separation are taking place in the nucleus, division of the cytoplasm starts. This is a simple constriction into approximately equal parts. Several instances of double nuclear division were found (fig. 23), and ordinarily the spindles lay parallel. In no case were more than two spindles found.

FORMATION OF GAMETES.—In old cultures the amoebae form gametes (fig. 69). The first stage in gamete formation is enlargement of the karyosome (fig. 28), which then fragments, the chromatin particles being extruded into the cytoplasm (figs. 29–32). These become collected into aggregates of chromatin surrounded by a clear area (fig. 30). The cytoplasm then contracts around these, so that a mass of small gametes is formed, each with an anterior nucleus (fig. 32). These gametes at first are very small and spherical in shape, with a poorly differentiated nucleus, which lies near the periphery. At this point the flagellum appears apparently growing out from the nucleus (fig. 33). I have concluded that the flagellum is located at the anterior end of the organism, since, whenever the organism moves the flagellum precedes it. Development of the flagellum is accompanied by increase in size of the gametes.

The gametes from many amoebae are released at the same time and form a swarm (fig. 69). In this stage they undergo a process which I believe to be conjugation. Two flagellates apparently conjugate to form a uniflagellate zygote. The stages of this process as found in prepared slides are shown in figs. 33, 69. I believe that the process is one of true conjugation rather than division, because it differs so radically from the stages of undoubted division which are described later. Although there is no conclusive evidence, I believe

that the zygote becomes uniflagellate by the fusion of the two gametic flagella. The change from the flagellate form to amoeba, or vice versa, can be induced by the addition of distilled water or Knop's solution to the culture, or by lowering its temperature.

Flagellate stage

Morphology.—The flagellates which arise from the amoebae are pyriform in shape and appear smaller than the amoebae. The younger flagellates differ somewhat from the older in size and shape, being larger on the average, and having a width of one-third the length of the body as compared with two-thirds in the older organisms. No differentiation into ectoplasm and endoplasm was observed.

The nucleus of the flagellate is like that of the amoeba, with a well defined membrane and central karyosome, and divides promitotically. The position of the nucleus in the cytoplasm is variable, generally being near the center of the organism, but some forms were found in which it had migrated into the anterior end. In all such cases the organisms were feeding very rapidly, and the flagella had become longer.

The single flagellum is about twice the length of the organism, and is situated at the extreme anterior end. It ends just inside the periphery in a deeply staining granule, the blepharoplast, which is connected with the karyosome by means of a rhizoplast.

Individuals were found with the nuclear membrane pulled out in the direction of the blepharoplast (fig. 37); also with a long flagellum and a nucleus lying close to the blepharoplast (fig. 57*a*). This increase in flagellar length is coincident with movement of the nucleus to a position near the blepharoplast. Judging from these observations, the blepharoplast may either be derived from the centriole or budded off from the karyosomic material around it. It arises from the karyosome and is always connected with it. After extension from the nucleus it takes up its position at the periphery of the cytoplasm coincident with the growth of the flagellum.

Under certain environmental conditions the flagellum is pulled back into the organism. This has been observed in living material, and the indications are that the material is returned to the nucleus. Reflagellation begins with a change of form (fig. 37).

The flagellate feeds in the same manner as the amoeba. Bacteria are always taken in at the posterior region, where they are digested in vacuoles (figs. 8–11).

BINARY FISSION.—The first step in binary fission is generally the division of the blepharoplast, although sometimes the development of the new flagellum precedes it. Next the nucleus divides mitotically, and last of all the cytoplasm divides longitudinally. Cytoplasmic division begins before the nuclear division at the anterior end and rapidly proceeds posteriorly. Late stages of division give the appearance of an elongate organism with an anterior and a posterior flagellum, but this is only a transitory state (figs. 40–46). When the division is complete the posterior ends round off and the daughter individuals move away from each other. In all cases the newer flagellum is less active than the older and becomes attached to débris, while the older moves with a characteristic jerky movement.

From observations made during the course of the investigation, it is apparent that the flagellates occur more commonly in the cultures than the amoebae. In a fresh culture made from spore cysts amoebae usually appear, but as the culture grows older the flagellates become more and more numerous.

Most of the amoebae in a culture can be transformed into flagellates in two hours by lowering the temperature. A little over an hour after a preparation has been made from a cooled culture, contracting amoebae with developing flagella are found in different places on the cover glass. These will be found to be more or less free from the substratum, and to be moving at the anterior end by a characteristic jerky movement, through a rather wide range of space. After the amoebae have been transformed into flagellates they send out and retract short pseudopodia almost continuously, and twist and turn, getting nearer all the time to the rounded up condition. When they reach a thick, long, pyriform shape they swim away with the narrow anterior end foremost (figs. 37–39).

In changing from the flagellate to the amoeboid stage, the organisms settle to the bottom, develop pseudopodia, retract their flagella, and assume the typical amoeboid form (fig. 47). This change can be induced by lowering the temperature or by mechanical disturbance. After such stimulation the flagellate begins to whirl

around until it seems exhausted, then settles to the bottom and assumes the amoeboid form.

Encystment

Encystment follows a period of rapid division, and appears to depend upon the heaviness of inoculation or upon the relative number of amoebae in the culture. The onset of the process is characterized by a cessation of division, as only an occasional division figure is found in preparations from cultures in which most of the individuals are encysting (fig. 70).

Organisms undergoing encystment are characterized by the entire absence of food vacuoles, by a rounding up of the cytoplasm, and by enlargement of the contractile vacuole with an increase in the rate of its pulsation (fig. 26). The protoplasm becomes dense, the contractile vacuole disappears, and a thin wall is formed around the organism. Later a second wall is formed within this, which is much thicker and contains no visible opening (fig. 34).

In some encystments a sporoblast is found filled with minute inclusions which may be "parasites" (fig. 36). Under proper conditions, such as the addition of fresh culture medium, these inclusions move about violently within the sporoblast, and finally rupture the cyst wall and are liberated (fig. 36). These inclusions appear to be similar to the dots seen in the plasmodia, nuclei, and chloroplasts of invaded tobacco tissues.

An amoeba that is emerging from the cyst may easily be recognized by the appearance of contractile vacuoles with a rapid rate of pulsation. The cyst wall disappears and a long pseudopodium is protruded. Following this the amoebae come together to form the plasmodium (figs. 60–64). This stage was observed in two cultures during January and April in 1924.

Plasmodium

The plasmodium appeared after the addition of fresh Knop's solution to cultures following encystment of the amoebae induced by evaporation. The amoebae which fuse to form the plasmodium are characterized by the presence of long stringy pseudopodia (figs. 60–64), relatively larger nuclei than those of the ordinary amoebae (fig. 62), the entire absence of food and contractile vacuoles (figs. 63,

64), and less differentiation between the ectoplasm and endoplasm (fig. 62). When pseudopodia of two of these organisms come in contact they fuse and the two amoebae flow together. By this process hundreds of amoebae run together to form a huge plasmodium (figs. 60–64). The plasmodium moves like a huge amoeba but more actively. The line of flow is generally in one direction, but the plasmodium may often change its course (figs. 65, 66).

The ectosarc is very clear and liquid, of unequal thickness over different parts of the organism, and generally abundant at the advancing margin. The nucleus of each amoeba that enters into the formation of the plasmodium retains its individuality, and no nuclear division has ever been observed, although there is a change just before the free spores are formed. In other words, each nucleus undergoes a reorganization before spore formation. The plasmodium takes in food by the flowing of the protoplasm around the object, but just prior to and during spore formation no food is ingested.

Immediately before the free spores are formed, the cytoplasm becomes clearer and more granular. The free spores are liberated in a row as the plasmodium moves along (fig. 67). Each free spore contains a single nucleus and a highly vacuolated cytoplasm (fig. 50). The nuclei of these spores enlarge, and chromidia are given off which form aggregates, around each of which a cyst wall is formed. Each free spore will produce three to fifteen of such sporocysts (figs. 54, 55).

Fig. 2 combines what I believe to be the sequence of forms in culture. Although I have obtained direct evidence for each sequence figured, further work may necessitate a number of minor changes. In this connection it should be noted that certain flagellates and amoebae of the types depicted in figs. 57–58*g* were observed in my cultures which cannot at present be fitted into any scheme of the life cycle. In considering the forms shown in fig. 2, it should be emphasized again that the only form which I have ever encountered in sections of the plant is the plasmodium (figs. 1, 3, 4, 59).

Possible relation to tobacco mosaic

The occurrence of the plasmodium of this organism in the cells of tobacco plants affected with mosaic-like symptoms raises the ques-

tion whether the organism or its inclusions or both are causally related to mosaic of tobacco. Only approximately twenty-five healthy and twenty-five diseased plants have been sectioned and studied so far. In none of the healthy plants was the organism found, nor was it obtained from them in culture; it was found in all of the diseased plants and also was obtained from them in culture.

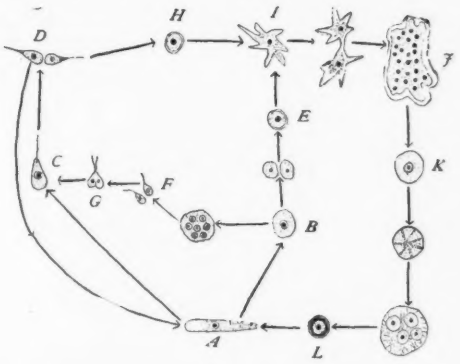

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FIG. 2.—Diagram of life cycle of *Plasmodiophora tabaci:* amoeboid stage (*A*) gives rise to other amoebae (*B*) by division, or is transformed directly into flagellate stage (*C*); amoebae (*B*) either encyst (*E*) or give rise to gametes (*F*) which fuse to form zygote (*G*); this gives rise to the flagellate (*C*) which gives rise to other flagellates by binary fission (*D*); these can either return to the amoeboid condition (*A*) or encyst (*H*); cysts (*E, H*) give rise to amoebae (*I*) which fuse to form plasmodium (*J*); plasmodium gives off free spores (*K*) which give rise to spore cysts (*L*); from these the amoebae (*A*) arise.

The fact that the plasmodium has not been found in plants free from mosaic-like symptoms is significant, although it does not prove that the organism is the pathogene.

The work done so far in culturing the organism and inoculating plants with these cultures is of a preliminary nature, and has not been critical enough to establish the causal relationship. Inoculations of healthy plants with cultures of the organism have produced the disease; the organism has been demonstrated in the tissue of the inoculated plants, and subsequently has been obtained from them in culture. The difficulty is, however, that these were not *single* pure

cultures, and that the possibility of contamination with a virus has not been eliminated. Two sets of experiments were conducted with nine plants each. In the first set, three plants were inoculated subepidermally with an agar-agar culture of bacteria obtained when affected leaves were placed in Knop's solution; three plants were inoculated with the culture of the flagellate stage of the organism, plus the bacteria which developed in culture from the plant tissue; and three plants were held as controls. Only the three plants inoculated with the mixed culture of flagellates and bacteria developed the disease. In the second experiment three plants were inoculated subepidermally with a mixed culture of bacteria and plasmodia; three were inoculated with a mixed culture of bacteria and flagellates; and three were held as controls. The plants inoculated with the plasmodium and those inoculated with the flagellates developed the disease twenty-one days after inoculation, while the controls remained healthy.

So far it has not been possible to grow the organism in cultures free of bacteria. If cultures are entirely free the organism does not live long. The bacteria seem to be a part of its food, and their relative abundance determines to a great extent the state in which the organism occurs in culture. Thus when bacteria become scarce in culture the flagellates and amoebae encyst; when they become abundant again the organisms excyst.

Work is in progress on the problems arising out of the findings reported in this paper.

Summary

1. A new species of a mycetozoan has been cultured from tobacco plants with mosaic-like symptoms.

2. Sections of leaves of tobacco plants affected with mosaic-like symptoms show the plasmodium stage of the organism within the plant cells. The peripheries of nuclei and of chloroplasts often show little dots which suggest the inclusions found in the cysts of the organism in culture.

3. In culture the mycetozoan has a flagellate and an amoeboid stage, and forms gametes, plasmodia, free spores, and cyst spores.

4. Certain inclusions are found in the cyst which may be "parasites" of the mycetozoan.

5. The mycetozoan described is provisionally placed in the genus *Plasmodiophora* and is named **P. tabaci.**

6. Amoebae and flagellates which differ somewhat from those of *P. tabaci* have been cultured in Knop's solution from tomato plants affected with mosaic, from potato plants affected with leaf roll, and from intestines of aphids feeding on diseased tobacco, tomato, and potato plants.

I wish to express my indebtedness to Professor G. N. CALKINS for many helpful criticisms and suggestions while this work was in progress at Columbia University. I am indebted to M. H. RUCKES for the photographs; to Miss D. DOWNIE for preparation of the drawings in figs. 1, 3, and 4; to Mrs. C. C. SPEIDEL for copying the line drawings; and to H. R. HALSEY.

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EXPLANATION OF PLATES XXXIV–XXXVII

PLATE XXXIV

FIG. 3.—Detail of plasmodium represented diagrammatically in fig. 1, drawn with camera lucida; ×about 1000.

FIG. 4.—Portion of huge plasmodium found in tissues of tobacco leaf: section cuts a vein obliquely and exposes a few spiral thickenings; plasmodium lies in phloem and adjoining tissues; some cell walls disappeared; engulfed in plasmodium are three host nuclei as well as chloroplasts in all stages of degeneracy; nuclei of plasmodium stand out clearly; drawn with camera lucida; ×about 1000.

FIGS. 5–58g.—All drawings made with camera lucida; ×1200.

FIGS. 5–11.—Organisms taken from one oil immersion field, showing change from flagellate to amoeba stage and method of feeding; figs. 5–7 are amoebae, and figs. 8–11 are flagellates taking in food; figs. 8, 10, flagellates dividing.

FIG. 12.—Amoebae in flowing (limax) stage.

FIG. 13.—Amoeba in floating form showing several small pseudopodia.

FIG. 14.—Amoeba showing pseudopodia produced by alternating flow.

FIG. 15.—Amoebae showing resting nucleus with central karyosome, clear space, and nuclear membrane with small amount of peripheral chromatin.

FIG. 16.—Amoeba rounding up preparatory to division.

FIG. 17.—Prophase showing elongated nucleus, vacuolated karyosome, and peripheral chromatin somewhat more evident than in resting stage.

FIG. 18.—Dumb-bell-shaped karyosome showing development of spindle.

FIG. 19.—Late prophase showing polar caps, granular chromatic polar masses, spindle fibers, and centrodesmus.

FIG. 20.—Early prophase showing constriction of nuclear membrane.

FIG. 21.—Anaphase showing chromatin masses moving apart.

FIG. 22.—Late anaphase showing condensation of chromatin to form daughter karyosomes.

FIG. 23.—Division of binucleate amoeba showing two parallel spindles.

FIG. 24.—Binucleate individual showing nuclear reconstruction.

FIG. 25.—Individual undergoing rapid division, showing division of daughter nuclei before constriction of cytoplasm is completed.

FIG. 26.—Encystment of amoeba, showing enlarged contractile vacuole characteristic of this stage.

Fig. 27.—Amoeba completely encysted.

FIG. 28.—Enlargement of karyosome preceding fragmentation.

PLATE XXXV

FIGS. 29–31.—Formation of gamete nuclei by fusion of extruded chromidia.

FIG. 32.—Later stages in gamete formation, showing breaking down of cell nucleus and formation of gamete nuclei.

FIG. 33.—Outgrowth of flagellum from gamete and subsequent fusion of gametes to form zygote.

FIG. 34.—Encysted amoeba showing thin and thick cyst wall.

FIG. 35.—Encysted amoeba showing thin cyst wall, and nucleus with large amount of peripheral chromatin.

FIG. 36.—Cysts with mature sporoblast containing inclusions (parasites?); cyst nucleus pressed flat against cyst wall.

FIG. 37.—Amoeba changing into flagellate stage, showing outgrowth of blepharoplast from karyosome of nucleus.

FIG. 38.—Flagellate stage showing completed flagellum connected with karyosome by means of rhizoplast.

FIG. 39.—Flagellate showing completed flagellum ending in blepharoplast.

FIG. 40.—Beginning of division of flagellate, showing division of blepharoplast while remainder of organism is still in resting stage.

FIG. 41.—Completed division of blepharoplast and flagellum, showing one daughter flagellum in active motion, other trailing it.

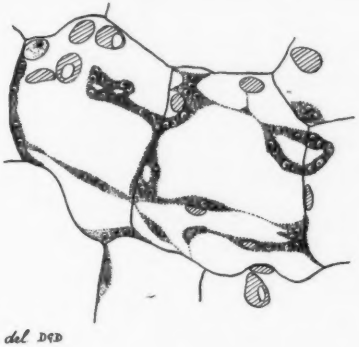
FIG. 42.—Beginning of cytoplasmic division; one of the two daughter blepharoplasts has a flagellum, other has not yet developed one.

FIG. 43.—Later stage in cytoplasmic division, with anterior ends of the two individuals pulled apart, giving appearance of an elongate individual with flagellum at each end.

FIGS. 44, 45.—Two flagellates with anterior ends of daughter individuals separated, showing beginning of nuclear division.

FIG. 46.—Later stage in division, showing completed nuclear division and cytoplasm constricting.

FIG. 47.—Organism that began division in flagellate stage and is completing it in amoeboid stage; nuclear division completed and cytoplasmic division begun.



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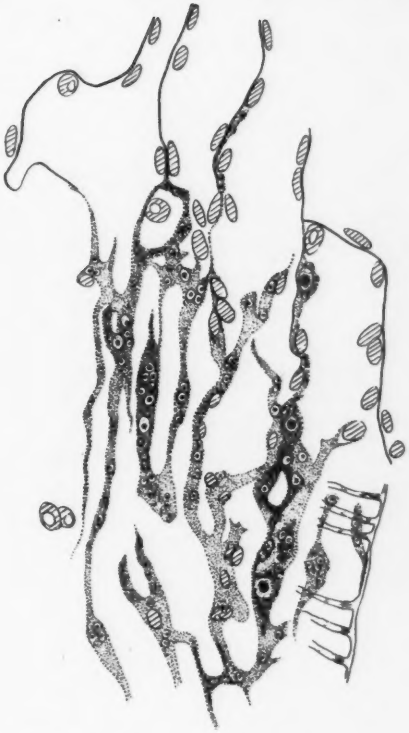

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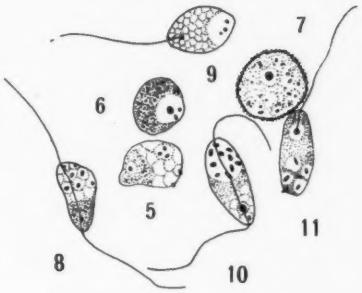
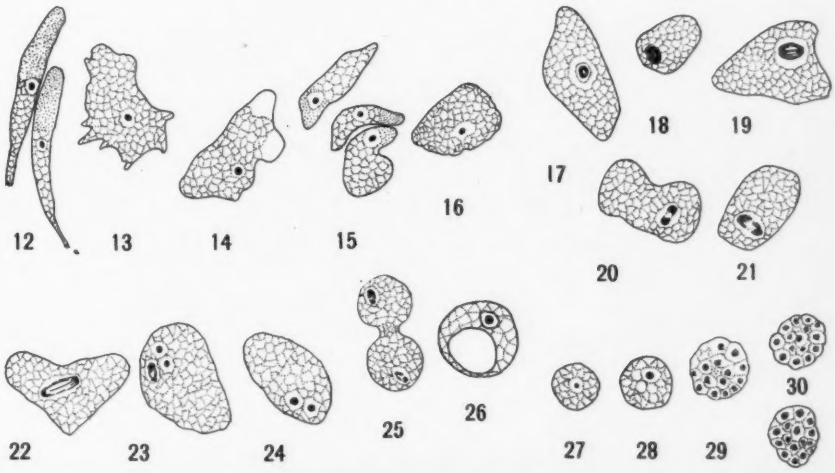
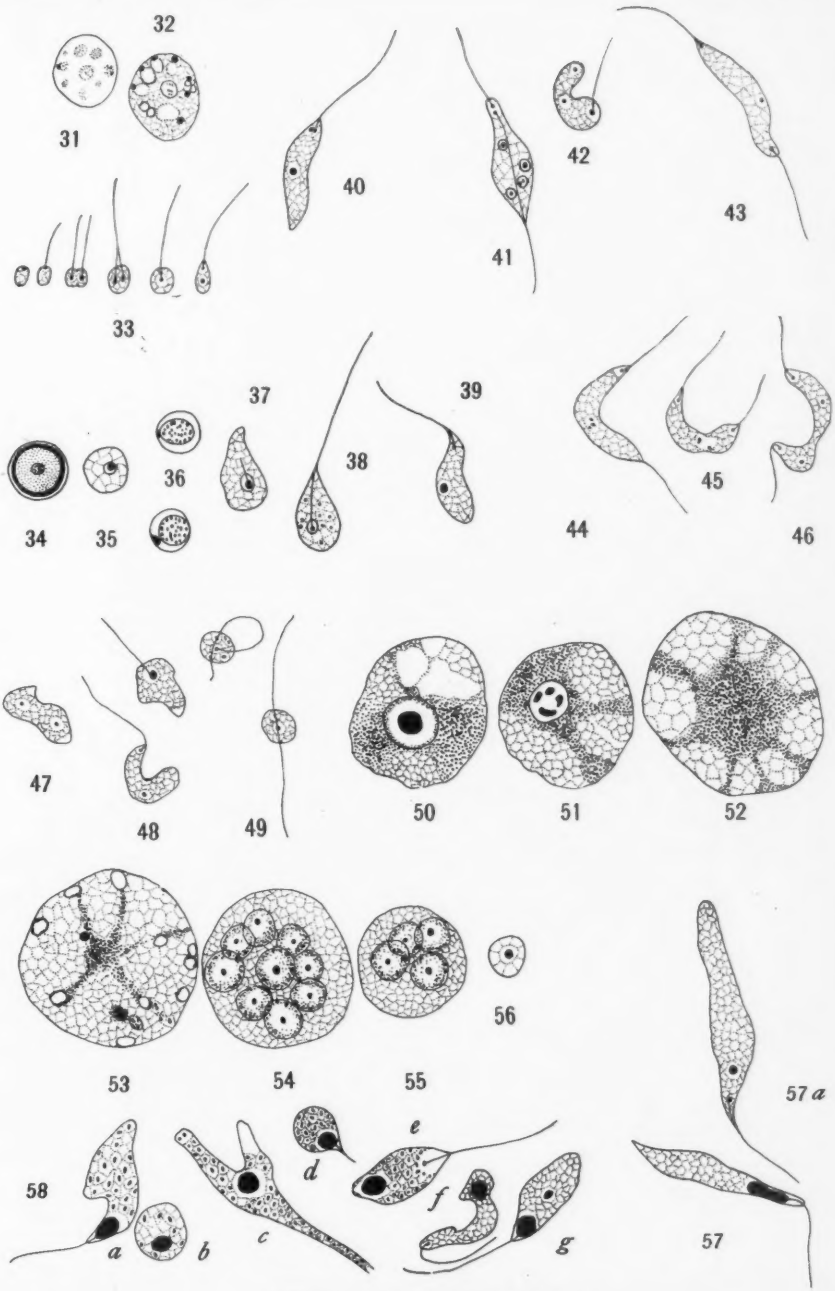




JONES on A MYCETOZOAN



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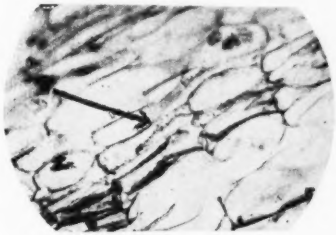

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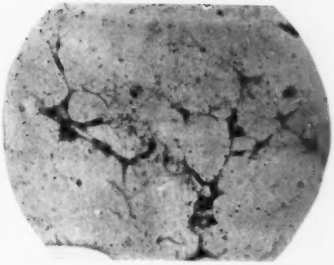

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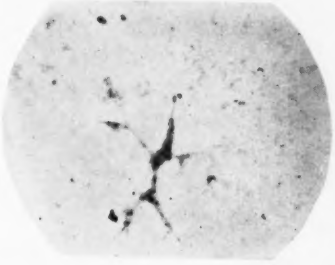

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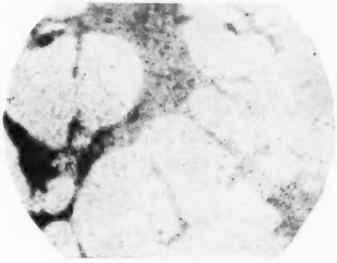

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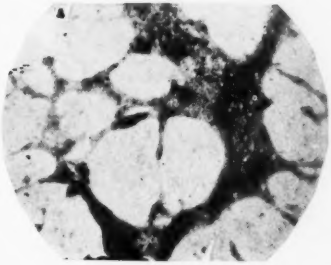

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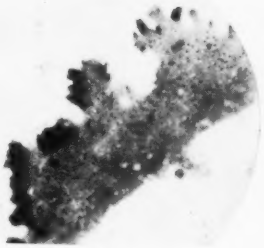

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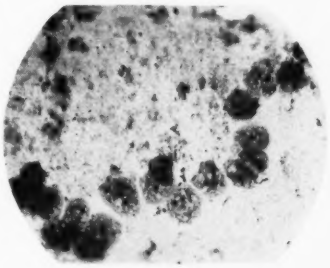
JONES on A MYCETOZOAN



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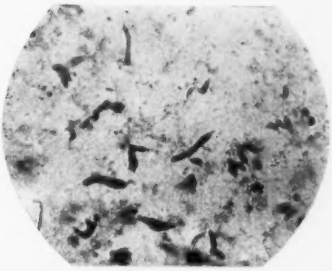

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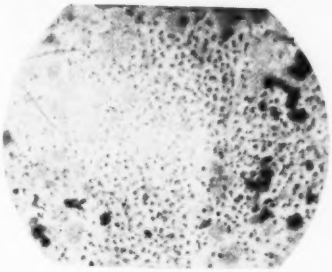

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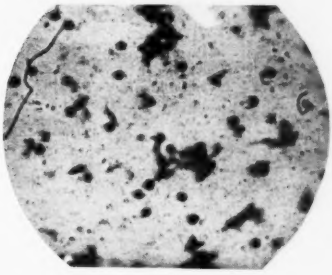

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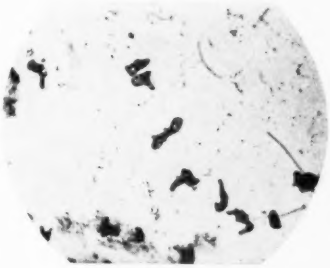

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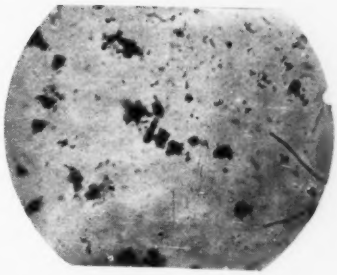

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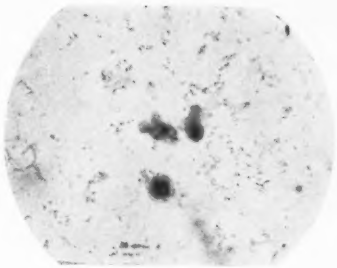

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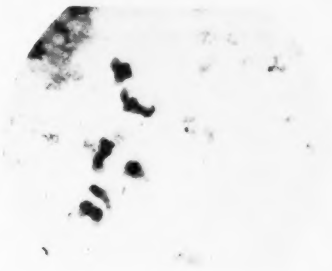

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FIG. 48.—Flagellates changing into amoebae, showing flattening out of organism, taking in of flagellum, and outgrowth of pseudopodium.

FIG. 49.—Encystment of flagellate, showing withdrawal of flagellum and rounding up of organism.

FIG. 50.—Free spore deposited by plasmodium, showing beginning of chromodial extrusion.

FIGS. 51, 52.—Breaking down of spore nucleus and extrusion of chromidia.

FIGS. 53–55.—Formation of sporocysts within free spore.

FIG. 56.—Mature sporocyst.

FIG. 57.—Nucleus at anterior end in act of forming blepharoplast.

FIG. 57*a*.—Nucleus moving posteriorly after forming blepharoplast.

FIG. 58*a*.—Amoeba changing into flagellate; note large size of nucleus.

FIG. 58*b*.—Amoeba encysting with same type of enlarged nucleus.

FIG. 58*c*.—Flowing amoeba.

FIG. 58*d–g*.—Development of flagellum.

FIG. 58*e*, *f*.—Nucleus in posterior end (condition causing this change not known).

PLATE XXXVI

FIGS. 59–75.—Photomicrographs;×about 350.

FIG. 59.—Section of root of tobacco plant, showing plasmodium in cortex.

FIGS. 60–64.—Fusion of amoebae to form plasmodia.

FIGS. 65–66.—Plasmodia.

PLATE XXXVII

FIG. 67.—Free spores deposited in a row by plasmodium.

FIG. 68.—Amoebae.

FIG. 69.—Gametes swarming and fusing.

FIG. 70.—Flagellates encysting and cysts.

FIG. 71.—Amoebae from tomato plants with mosaic symptoms.

FIG. 72.—Amoebae cultured from intestines of an aphid feeding on tomato plant with mosaic symptoms.

FIG. 74.—Amoebae cultured from potato plant with leaf roll symptoms.

FIG. 75.—Amoebae cultured from intestines of an aphid feeding on potato plant with leaf roll symptoms; figs. 60–75 from material grown in Knop's solution.

FLORA OF AN ILLINOIS COAL BALL

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 349

FREDDA DORIS REED

(WITH PLATE XXXVIII AND ONE FIGURE)

Introduction

The Carboniferous flora of Europe and England is quite well known, as the result of numerous investigations conducted for the most part within the last fifty years. Until recently (3), however, material for the study of the Carboniferous flora of America was not found in great quantities, except in the form of impressions. During the last year Dr. A. C. NOÉ, of the University of Chicago, has investigated coal fields of Illinois, Iowa, and Kentucky, and has collected an immense amount of material in the form of petrifactions, or coal balls as they are called technically, all of which contain sectionable plant tissue in varying quantities and in varying degrees of preservation. With this abundance of material, a Carboniferous flora of America as rich and diversified as that of Europe should be reconstructed. It seems advisable, therefore, to publish accounts of the various plant types as they are uncovered.

The plants described in this paper were found in one coal ball which came from the Calhoun Coal Company, Calhoun, Richland County, Illinois. The coal seam is no. 15, which belongs to the McLeansboro formation or the Upper Pennsylvanian of Illinois.

The ball measured approximately 5 cm. in diameter, and an analysis showed that it was composed largely of calcium carbonate. From the ball eighteen sections were made, all of which show plant tissue, but in many cases the tissues were too fragmentary and isolated to be of any value. Of the plant structures that could be identified, however, there was a remarkable variety ranging through the Pteridophytes and lower Gymnosperms.

Of the stems found only the stele was described, for the cortex was either not preserved at all or was too poorly preserved to permit description. Furthermore, only transverse sections were made, but

in most cases they were cut in a slightly oblique manner or were so thick that the markings on the walls of the tracheids were discernible.

Calamites

In a number of sections there is a portion of wood possessing an extensive secondary growth which has been identified as belonging in the *Calamites* group. This bit of wood appears to be one-fourth of a transverse section of a stele whose diameter would measure about 1.5 cm. As in most *Calamites* stems, there is very little pith present, and in this case there is no tissue preserved outside the secondary wood. The primary wood and the wood rays are quite well preserved, and show the anatomical features of certain *Calamites* species.

One of the most prominent characteristics of *Calamites* is the carinal canal found at the apex of each of the xylem wedges, and formed by the disorganization of the protoxylem; such a canal is quite distinctly seen in this specimen. In some of the bundles the canal borders directly on the pith; in other instances there is evidence of a row of small cells surrounding it, which are probably the remaining cells of the protoxylem, but in either case the development of the metaxylem must have been entirely centrifugal. Abutting on the metaxylem is the secondary wood, whose radial thickness consists of some eighty elements.

Extending between the xylem wedges are the parenchyma rays, composed of exceedingly wide and thin walled cells. These tapering rays are gradually shut off by the interfascicular wood.

The formation of a carinal canal, the absence of centripetal primary xylem, and the character of the principal wood rays are all peculiar to the *Calamites communis* (Binney) type (5), which appears so frequently in the English Coal Measures and is also the common type found in Europe. Since this American type possesses these same anatomical features the same name has been applied to it.

Sphenophyllum

Sphenophyllum has been found in the Devonian, appears throughout the Coal Measures, and extends into the Permian. Considering such a wide geological range, the anatomy of the stem is

remarkably uniform, for only two species based upon stem structure have been described: *Sphenophyllum insigne* and *S. plurifoliatum*, the former a Pre-carboniferous species and the latter common throughout the Coal Measures. The specimen found in this American coal ball, in so far as it is possible to describe it from a transverse section, agrees in every particular with that of *S. plurifoliatum*. SCOTT (**6**) has associated this form with that of the contemporary *S. myriophyllum*, known only from impressions.

In many of the sections there appear portions of the stele more or less complete, occasionally with bits of the cortex attached. Fig. 3 shows a section in which the stele is almost entirely preserved. Here it measures about 3 mm. in diameter. The photograph shows the solid strand of primary wood of triarch structure which was centripetally developed, for at the apex of each angle may be seen cells of much smaller caliber, which are the elements of the protoxylem. The organization of the secondary wood is of that type peculiar to this species: radiating out from the angles of the primary xylem are the tracheids of the fascicular wood, and between these groups are ones of larger tracheids, regularly and radially arranged, which are known as interfascicular wood. The tracheids of the primary and of the secondary wood are marked with multiseriate pits on the radial as well as on the tangential walls.

Between the truncated angles of the tracheids of the secondary wood are little groups of thin walled cells. From longitudinal sections these cells have been found to be vertical strands of parenchyma forming the medullary rays (**7**). The presence of these thin walled cells, making the medullary rays, and the absence of the carinal canals formed as in *Calamites* by the disorganization of the protoxylem, are the characteristics that distinguish this from the *Sphenophyllum insigne* type.

Bothrodendron

As long ago as 1833, LINDLEY and HUTTON (**4**) identified some impressions as *Bothrodendron*, but it was not until 1890 that the internal structure was known. At that time LOMAX found a stem that had the external appearance of *Bothrodendron*, and possessed the anatomical features of *Lepidodendron mundum* previously described

by WILLIAMSON (12), and so established *Bothrodendron mundum*. WILLIAMSON also described a species of *Stigmaria* which is apparently the underground or creeping portion of the stem.

In 1908 WATSON (9) gave a detailed account of a cone which, from the anatomy of the axis, he was able to relate to this species, and also determined that it was identical with a Lepidodendraceous cone figured and described by WILLIAMSON in 1880 (11). Furthermore, detached sporangia bearing spores described by WILLIAMSON (10) in 1878 belonged to this same species. Thus almost the entire plant has been described, and it is found to be one of the most ancient of Lycopods, for impressions of it have been found in the Devonian.

In this coal ball there were portions of the vegetative structure, a detached microsporangium and a megasporangium, both containing spores, and a number of isolated spores. The vegetative structure is a transverse section of a stem, and is illustrated in fig. 4. Judging from the size it must have been a stem tip, for the entire stem, including the cortex, measures only 2 mm. in diameter, while the diameter of the stele is approximately 0.8 mm. The stele is of the protostele type, but shows evidence of transition to the siphonostele condition, which is the prevailing type in the larger stems of *Bothrodendron*. The primary wood is composed of very large elements, exhibiting reticulated and pitted thickenings. External to the primary wood are the small cells of the protoxylem. There is no secondary growth.

The inner cortex has almost entirely disappeared, but the outer cortex is fairly well preserved. These cells are somewhat heavier walled than the few remaining cells of the inner cortex, and are irregularly placed.

The microsporangium shows the attachment to the sporophyll. One section (fig. 5) is almost a median cut of the sporophyll, for it shows the tracheids with spiral and scalariform thickenings; it is not quite median, however, for only a portion of the ligule appears. Near the base of the ligule, or rather near the ligular pit, may be seen some tracheids directed from the normal course of the bundle toward the ligule. This is not necessarily an indication that the ligule was supplied with a vascular strand, but that here is a situa-

tion analogous to that in *Selaginella*, in some species of which cells beneath the glossopodium become thickened, and in the mature condition are transformed into short tracheids, so that the leaf trace appears to be enlarged beneath the ligule (**2**).

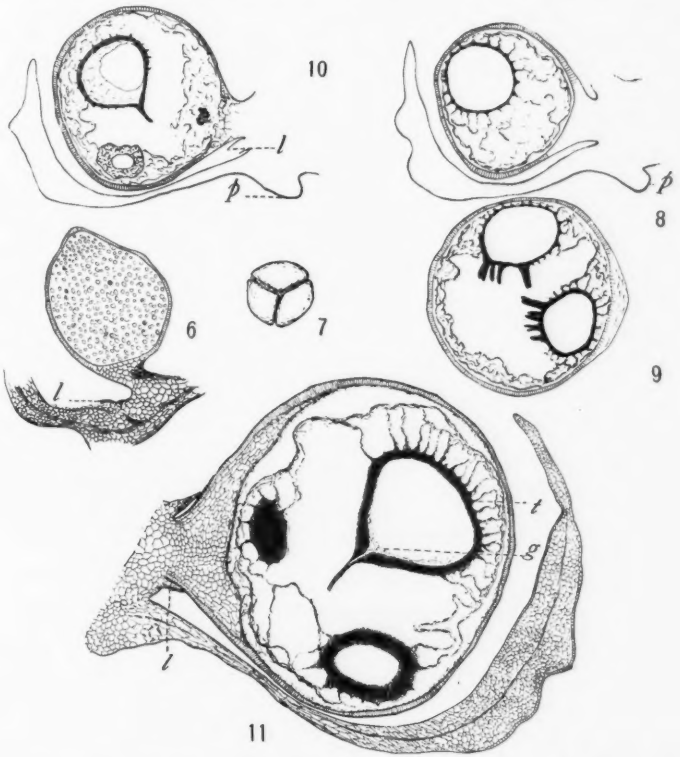
The sporangium is roughly spherical in form, but somewhat higher than it is wide, and measures about 2.5 mm. in diameter. The wall consists of one row of radially elongated cells. Inclosed in the sporangium are a great number of microspores in the tetrad stage, as illustrated in figs. 6 and 7. The diameter of the tetrads of spores varies from 0.05 to 0.06 mm. A rough calculation of the number of spores gives 80,000, which is a conservative figure. The microspores show no markings or sculpturing on the walls.

The section of the megasporangium is much more satisfactory than that of the microsporangium, in that it is much more complete. The material from which figs. 8–10 were made is not now available, for these are camera lucida drawings made during the preparation of the sections, and the material is now ground away.

The sporophyll does not show the attachment to the cone, but was apparently broken off quite near it. Fig. 10 shows the lobe on the lower part of the sporophyll, and a portion of the ligule (*l*), both of which features are described by WATSON (**9**). The course of the vascular strand is indicated by the large scalariform tracheids, which are found to extend about four-fifths the length of the lamina. This, however, does not mean that the vascular strand terminated here, but merely that the section is so cut that the exact termination does not show.

The megasporangium is more nearly spherical in form than the microsporangium, and is larger, being about 3 mm. in diameter. The pedicel of the sporangium is attached to the horizontal portion of the sporophyll, it is quite narrow and very short, and expands into the sporangium, its epidermal cells becoming the wall cells of the sporangium. The wall of the sporangium is composed of one layer of radially elongated cells as in the microsporangium. In some places, however, there are indications of other wall cells, which would imply that there may have been other layers of wall cells, and that most of them have been resorbed by the functioning megaspores.

Four megaspores were observed, three of which appear in fig. 11. The fourth one disappeared in the preparation of the section. One



FIGS. 6–11.—Fig. 6, microsporangium showing portion of sporophyll, course of vascular strand, ligule, and tetrads of spores; ×10. Fig. 7, tetrad of microspores; ×230. Fig. 8, diagram of megasporangium showing sporophyll with lobe or projection of lower side, and one of the four megaspores; ×9. Fig. 9, diagram of megasporangium showing two megaspores; ×9. Fig. 10, diagram of megasporangium: *l*, ligule; *p*, lobe on sporophyll; ×9. Fig. 11, megasporangium with three of the four megaspores: *v*, vascular strand; *l*, ligule; *t*, tapetal plasmodium; *g*, female gametophyte; somewhat diagrammatic; ×16.

of the most prominent features of the megaspores is the thick outer spore coat, reminding one of the spiny exospore of living species of *Selaginella*. The method of the formation of this spore coat is sug-

gested in fig. 11; the spores are surrounded by a protoplasmic mass which apparently is homologous with the tapetal plasmodium of living Pteridophytes, as *Salvinia* and *Selaginella;* from this mass strands of protoplasm extend to the periphery of the sporangium, indicating that the outer spore coat is laid down by the tapetal plasmodium.

Some of the megaspores contain some tissue, probably that of the female gametophyte. If one assumes that the development of the megaspore parallels that of the megaspores of *Selaginella*, then the female gametophyte at this stage would be either in the uninucleate stage or in the subsequent free nuclear condition, which condition seems to be confirmed by the fragile appearance of the contents of the megaspore in the megasporangium, as well as in many of the isolated megaspores, one of which is shown in fig. 13.

A comparison of the fruiting and vegetative structures of *Bothrodendron* and *Selaginella* suggests a very close relationship between the two groups. *Bothrodendron* is like *Selaginella* in the possession of a creeping stem, a ligule, and the development of the spores. The widest divergence is probably in the anatomy of the stem, for there is nothing in this ancient Lycopod that approaches the organization of the lacunar spaces found in *Selaginella*. The imperfect preservation of the inner cortex of *Bothrodendron*, however, in contrast with the outer cortex, is a condition which is quite regularly found in Paleozoic Lycopods, and may partly be due to imperfectly and irregularly formed intercellular spaces. BOWER (1) reports lacunar spaces with very well developed trabeculae in the axis of *Lepidostrobus Brownii*, concerning which he writes, "It will be seen that these trabeculae, originating as they do from the irregular sheath surrounding the bundle, are very similar to the trabeculae of *Selaginella*." *Selaginella*, therefore, is not peculiar in the possession of these features, but rather possesses well developed and highly specialized forms of lacunae and trabeculae, the prototypes of which may be found in the primitive Lycopods.

Lyginopteris

In addition to the preceding, there is yet another woody stem whose identity is more uncertain than those previously described,

although the evidence seems to place it in the lower Gymnosperms. The stem or stele is illustrated in fig. 14. As may be seen, it is not round but slightly flattened in outline, its larger diameter measuring 4 mm. In the center there is a group of relatively thin walled cells, rather sharply delimited from the cells of the primary xylem, making the medulla or pith. This central strand or pith is surrounded by the primary xylem, which is organized in six or seven bundles. The tracheids of the primary xylem are marked with multiseriate pits on all walls alike, and they inclose smaller cells with spiral and scalariform thickenings of the protoxylem. These smaller tracheids are situated nearer the periphery of the primary xylem than the pith, but in any event the bundle is mesarch. The development of the primary wood was so extensive that it shut out the principal rays, and formed a continuous zone of wood around the pith.

Bordering on the primary is the secondary wood, whose radial thickness varies from five elements on the shorter radius to eight on the longer radius. Like the tracheids of the primary wood, these have multiseriate pits, but unlike the primary wood, the pits are confined to the radial faces.

Outside the zone of secondary wood the only tissue remaining is that of the leaf traces. The section is so thick and the preservation is so poor that it is impossible to orient the elements of these traces, but the gross topography shows that in general those traces which border the secondary wood are united, while those farther away from the stele have separated and are double. None of the leaf traces shows any secondary wood.

The double leaf traces, the type and disposition of the thickenings of the secondary wood, and the mesarch character of the protoxylem are all gymnospermic features, and may be found in the Lyginopterideae (**8**), so that one could not be far wrong in applying the generic name *Lyginopteris*. As for a specific name, it is hoped that further investigation will bring to light sufficient additional information to attach one.

Discussion

The reconstruction of any particular fossil plant must necessarily be a gradual process, since it is only rarely that the stem and roots or stem and leaves are found attached; or again, it is only in excep-

tional cases that impressions can be related to internal structure. For example, both *Calamites* stems and roots were known for some time before a specimen was found which made it possible to relate the two, and so the stem was given the name *Calamites*, but the root was described under the term *Astromyelon;* likewise, the leaves were known as *Annularia*. Even now that *Calamites*, *Astromyelon*, and *Annularia* are known to belong to one and the same plant, the terms persist more or less. Such a process, while obviously quite necessary, has resulted in a multiplicity of terms which are somewhat confusing. It was partly because of this situation that the use of new terms was avoided in this paper.

Again, the information concerning these plants, while sufficient for placing them in certain definite groups, is really quite meager; nothing is known of the external appearance, of the underground portions, of the leaves, and, except in the case of *Bothrodendron*, of the fructifications. Until these features are known and the plants can be definitely established, it seems best to resort to the nomenclature already in use.

Summary

In the coal ball described were portions of plants that have hitherto been known from the Carboniferous of America only as impressions; they are *Calamites*, *Sphenophyllum*, *Bothrodendron*, and *Lyginopteris*. Only transverse sections of the steles of *Calamites*, *Sphenophyllum*, and *Lyginopteris* were found. Belonging to *Bothrodendron* are transverse sections of a stem tip, a megasporangium and microsporangium both attached to sporophylls and containing spores, and isolated megaspores. In the microsporangium the spores are in the tetrad stage, while in the megasporangium four megaspores were observed. The most striking feature in the comparison of *Bothrodendron* and *Selaginella* is the similarity of the two, which indeed compels the conclusion that *Bothrodendron* is the progenitor of the living species of *Selaginella*.

Acknowledgment is due to Dr. A. C. Noé, who furnished the material and under whose direction the investigation was made.

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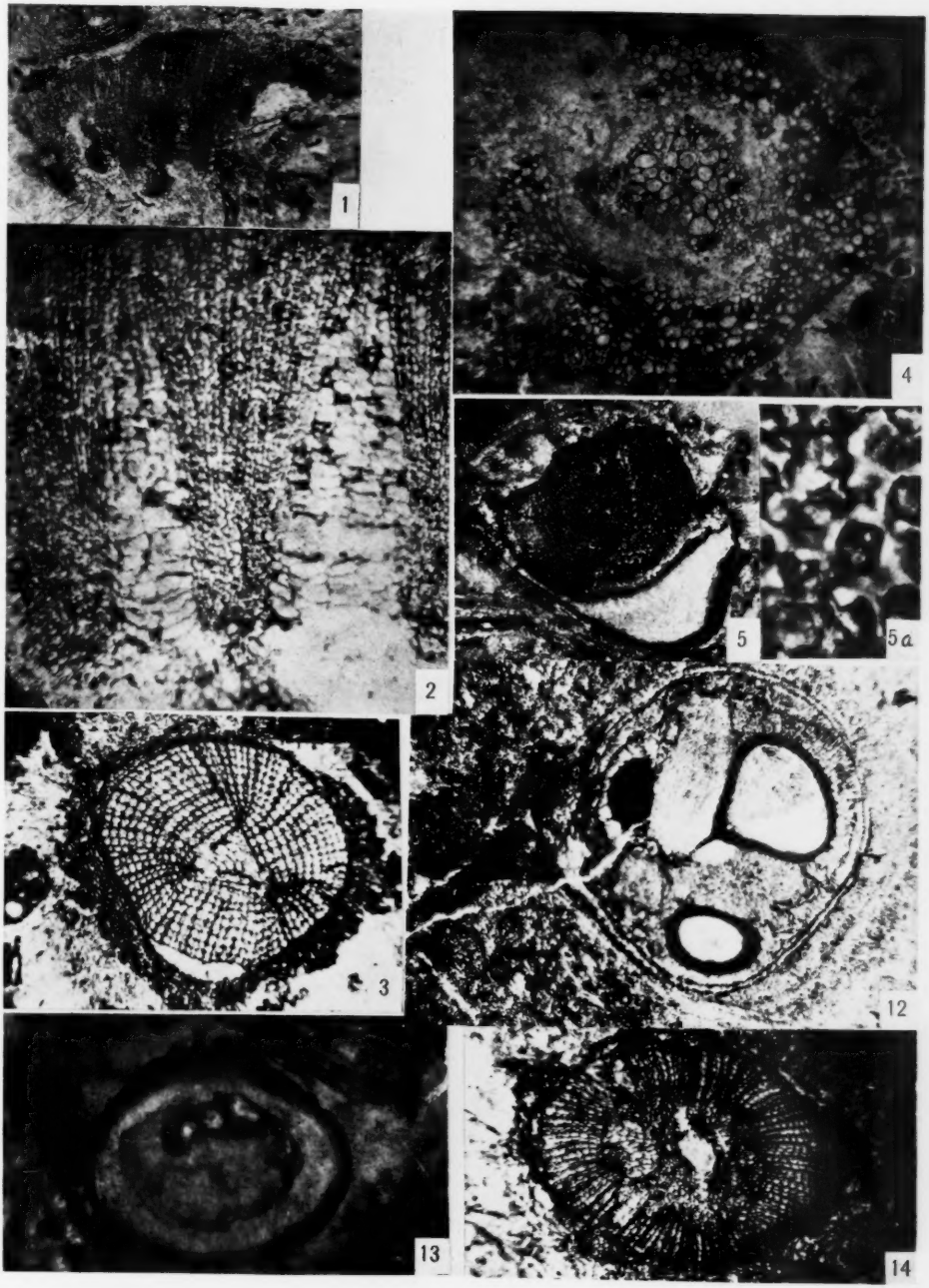

REED on COAL BALL

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EXPLANATION OF PLATE XXXVIII

FIG. 1.—Portion of transverse section of stele of *Calamites;* ×10.

FIG. 2.—Detail of one of bundles from fig. 1, showing few pith cells, carinal canal, extensive development of secondary wood, and primary wood rays; ×16.

FIG. 3.—Transverse section of stele of *Sphenophyllum;* ×12.

FIG. 4.—Transverse section of stem tip of *Bothrodendron: oc*, outer cortex; *ic*, inner cortex; *px*, protoxylem; ×28.

FIG. 5.—Section of microsporangium containing tetrads of microspores; ×12.

FIG. 5*a*.—Photomicrograph of tetrads of spores shown in fig. 5; ×150.

FIG. 12.—Photomicrograph of megasporangium containing three megaspores; ×16.

FIG. 13.—One of isolated megaspores containing some tissue; ×32.

FIG. 14.—Transverse section of stele of *Lyginopteris;* ×12.

CURRENT LITERATURE

BOOK REVIEWS

Plant pathology

Before reviewing some new books in this field, reference should be made to new editions of well established ones, for these have to some extent passed the test of time. The leading one is SORAUER'S[1] *Handbuch*, the most comprehensive of plant pathology texts. It is suggestive that the first two volumes are in the fifth edition and the other three in the fourth. The book is a dynamic factor which no pathologist can ignore. The merit of the first volume is its comprehensive point of view and its great fund of information; its weakness lies in its diffuseness. The latter probably is inherent in the subject matter, and is due in part to the fact that we are so little acquainted with the normal structure, physiology, and ecology of each of the numerous species of plants. The task of the plant pathologist is far more difficult than that of the human pathologist, because the field of the latter is much more restricted. There is an increasing use of American literature in the new editions, and consequently the book is becoming more useful by giving more truly the present status of phytopathological knowledge.

KÜSTER'S[2] *Pathologische Pflanzenanatomie* in its third edition is a complete revision, and maintains its leadership in the field of pathological plant anatomy. The presentation is so thorough and exhaustive that it is of use, not only to those interested in abnormal anatomy, but also to workers in the field of normal plant anatomy. The book consists of a special and a general part. In the former are considered (1) marbling, (2) etiolation, (3) hyperhydric tissues, (4) wound tissues and regeneration, and (5) galls. In the general part are taken up (1) histogenesis, (2) developmental mechanics, and (3) ecology of pathological tissues. KÜSTER's book has exerted a very considerable influence upon American plant pathologists, whose contributions in turn are increasingly finding their way into his admirable book.

Plant disease fungi, by STEVENS,[3] as its title indicates, deals mostly with fungi which cause plant diseases and very little with diseases. It is essentially

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[1] SORAUER, P., Handbuch der Pflanzenkrankheiten. Vol. I, 5th ed. 1925; Vol. II, 5th ed. 1925; Vol. III, 4th ed. 1923; Vol. IV, 4th ed. 1925; Vol. V, 4th ed. 1925. Berlin: Paul Parey.

[2] KÜSTER, ERNST, Pathologische Pflanzenanatomie in ihren Grundzügen. 3rd ed. pp. xii+558. *figs. 285.* Jena: Gustav Fischer. 1925.

[3] STEVENS, F. L., Plant disease fungi. pp. 469. *figs. 407.* New York: Macmillan Co. 1925.

a condensation of his previous book, restricting its scope to the more important pathogenic fungi which occur in the United States. The keys have not been made as workable, however, as one might hope for. It is true, of course, as STEVENS stated in his earlier book, that the "present unsatisfactory condition of taxonomy of the fungi, loose and imperfect description of species, disregarded generic limitations" make it difficult to produce workable keys. For these reasons it is all the more important that an author should strive for a certain amount of consistency of terminology in compiling a book of this nature. On page 116, in the key to the Phacidiaceae, the character "stroma" is used to separate the genera *Keithia* and *Coccomyces* from *Rhytisma*, the former lacking a stroma. On page 119 the legend of figure 115 is as follows: "An ascocarp of *C. hiemalis* on its thick stroma." Slips of this type detract from the usefulness of the book.

It is regrettable that the nomenclature of the bacteria was disturbed. The juggling of names of the Schizomycetes is of doubtful value at present, especially for beginners. It is especially regrettable that each shift must carry with it the name of the juggler. Of course, the outcome in the long run may be fortunate, because indulgence in this procedure will not stop until the situation becomes an unbearable nuisance. It is unfortunate that the older literature used in compiling the book is not cited as in the earlier volume. The book is printed on good paper and the figures show considerable detail.

In their laboratory outlines, WHETZEL, HESLER, GREGORY, and RANKIN[4] depart from the traditional American point of view of phytopathology, by attempting a classification of plant diseases on a pathological rather than a mycological or host basis. This is a welcome departure, and is a hopeful sign that possibly in the future, courses in plant pathology will deal with the subject matter of phytopathology, that is, plant responses. In the past fungi have been the central theme and plant responses merely interesting or necessary secondary considerations. There are difficulties in any system of classification. The system adopted in this manual is no exception. The difficulty in using necrotic, hypoplastic, and hyperplastic as categories in classifying diseases lies in the fact that the cellular responses may be of one type, whereas the response of the plant as an organismal unit, which after all is the essential entity from the theoretical and practical point of view, may be quite different. Thus in club root of cabbage the cellular response is hyperplastic, whereas the response of the plant as a whole is hypoplastic and necrotic.

The preface of the book contains a sentence in which we have the teaching philosophy of WHETZEL. He states: "Although the acquisition of a body of facts is an important and necessary part of the work in such a course, a more vital feature is the training in logical methods of acquiring them." The manual lives up to this high purpose to a remarkable extent.

[4] WHETZEL, H. H., HESLER, L. R., GREGORY, C. T., and RANKIN, W. H., Laboratory outlines in plant pathology. pp. 231. Philadelphia and London: W. B. Saunders Co. 1925.

WHETZEL'S manual is in part the result of a pedagogical experiment which he has been carrying on for some time. Simplification of the data of a science for pedagogical reasons always carries with it potentialities of danger. The phenomena of plant diseases, like many others in biology, cannot always be put into hard and fast logical compartments. The manual bristles with new words and phrases. In coining hard and fast definitions care should be used. WHETZEL takes a forward step in differentiating between the concepts "parasite" and "pathogene," but it is questionable whether the terms "pathogenesis" and "saprogenesis" have been wisely chosen. If pathogene means an "organism capable of causing disease," pathogenesis should mean the process of causing disease and saprogenesis the process of causing decay. These terms are not necessarily mutually exclusive, although they are so used in the manual. In the manual, according to the glossary, pathogenesis is taken to be that portion or phase of the life-cycle of a pathogene when it is associated with a living "suscept," whereas saprogenesis is taken to be the portion or phase of the life-cycle of a pathogene when it is not in association with the "suscept." These definitions in turn are based upon the coining of a new term suscept, and the dropping of the current botanical usage of the term life-cycle and adopting in its place that used by some workers in the fields of bacteriology and public health.

The book contains a wealth of information which is exceedingly useful to the teacher. For the beginner it possibly contains too much information. The student often is told in great detail to *observe* things which are minutely described in the manual, so that there is danger that he will develop habits of depending upon the manual and neglecting study of the material itself.

Imprisonment in a camp in Egypt during the war gave MORSTATT[5] an opportunity to produce an extremely worthwhile book. It lends itself well to use as a textbook for introduction into the theoretical and practical aspects of plant pathology. The book is only 159 pages long and necessarily sketchy. Chapter I, Die Erkennung der Pflanzenkrankheiten, takes up (1) the symptoms, and (2) methods of investigation and descriptions of diseases. Chapter II, Krankheitslehre, is extremely interesting and stimulating. It takes up (1) the concepts and nature of plant diseases, (2) pathological plant anatomy, and (3) pathological plant physiology. Chapter III, Die Ursachen der Pfanzenkrankheiten, presents in Part I (1) the general aspects of such topics as parasitism, symbiosis, specialization and transmission; followed by a synoptic discussion of (A) pathogenic plants, and (B) pathogenic and injurious animals. Filterable viruses as causative agents of plant diseases are treated in an appendix to this chapter. In the second part of Chapter III, there is a sketch of the non-living factors which cause diseases. Chapter IV, Pflanzenschutz, gives a brief summary of control measures. The book is a very successful attempt to present plant pathology as a unified field of applied botany.

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[5] MORSTATT, H., Einführung in die Pflanzenpathologie. V. pp. 159. *figs.* 4. Berlin: Gebrüder Borntraeger. 1923.

OWENS,[6] in his laboratory outlines, uses suggestive questions to stimulate the student. The outlines, however, suffer from brevity. OWENS very clearly conceives plant pathology as an applied field of botany, and consequently about three-fourths of his text is devoted to discussion of plant disease control. Under this topic he takes up the biological principles (such as parasitism and immunity) which have bearing upon control. Although there is recognition of the fact that plant pathology is more than a branch of mycology, the book is permeated by a mycological atmosphere. For each disease presented there is given a series of quotations from the relevant literature, saving much time for the student. It gives him some experience in reading the literature first-hand, and yet spares him the task of working through the vast amount of irrelevant and repetitious material found in many publications on plant diseases.

CHUPP,[7] has written a much needed book on the diseases of truck and garden crops. Emphasis is given to discussion of symptoms and control measures. The causal aspects, however, are not ignored. The book fits well into the Rural Manual Series, and will be extremely useful to growers and to extension workers. Students in plant pathology will find it a handy reference book. An enormous amount of work has been necessary to compile the diffused and widely scattered literature bearing upon diseases of vegetables.

CUNNINGHAM'S[8] *Practical bacteriology* is intended primarily for students of agriculture. It is a small book but is well written. It will prove useful primarily to bacteriologists. Chapter V (five pages) is devoted to the bacteriology of plant diseases.—G. K. K. LINK.

Biology of lichens

An interesting book on the biology of lichens has been written by TOBLER.[9] The material is presented under the following headings: (1) development and growth, (2) physiology, (3) ecology, and (4) symbiosis. TOBLER champions the concept "lichens." He believes that it is a useful concept and that its retention will stimulate morphological, physiological, and taxonomic studies of the component algae and fungi. TOBLER contends that a "lichen" is a new organism which has arisen out of the symbiotic relationship of an alga and a fungus, this organism typically being characterized by a new and distinct form, the lichen thallus, and even more significantly by a new and typical metabolism, the formation of lichen acids. Furthermore, this typical metabolism occurs only in

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[6] OWENS, C. E., Principles of plant pathology. A text and laboratory manual (mimeographed). Part I, pp. 126. Part II, pp. 288. Edwards Brothers, Ann Arbor, Michigan. 1924.

[7] CHUPP, C., Manual of vegetable-garden diseases. pp. xxiv+647. *figs. 155*. New York: Macmillan Co. 1925.

[8] CUNNINGHAM, A., Practical bacteriology. pp. vi+188. *figs. 14*. Edinburgh: Oliver and Boyd. 1924.

[9] TOBLER, F., Biologie der Flechten. pp. vii+265. one colored plate. *figs. 67*. Berlin: Gebrüder Borntraeger. 1925.

those lichens in which the symbiotic relationship has led to the typical morphogenic result. He contends that lichens are a group in active evolution, many members not yet having reached that level of symbiosis which is characterized by new and distinct form and metabolism. He takes decided but good tempered issue with FINK's suggestion that the concept "lichens" be dropped and the lichens be placed with the fungi.—G. K. K. LINK.

NOTES FOR STUDENTS

Filamentous algae of Iowa.—TIFFANY[10] has published a very full account of the filamentous algae collected in northwestern Iowa, illustrated by 16 plates. The collections were made briefly during late spring, summer, and early autumn, so that the whole growing season was represented. The total number of forms recorded is 200, the Myxophyceae including 35 and the Chlorophyceae 165. The Oedogoniaceae are particularly well represented, and are presented in more detail than the other groups, including 75 forms, with keys and plates in addition to the descriptions. The region of collection was in the "northwestern corner" of the state, where a summer laboratory is maintained by the University of Iowa. Within a short distance of the laboratory there are numerous lakes, swamps, streams, and drainage ditches, presenting a diversity of habitats rich in fresh water algae.—J. M. C.

Mosaic diseases.—FERNOW[11] has published some important results of an investigation of mosaic diseases. He conducted inoculation experiments on 19 host species, 15 of which were Solanaceae. All but one of these species proved to be susceptible to one or more of the 8 mosaics found to be distinct. These mosaics were distinguished by the species attacked, and by the different symptoms when inoculated on the same species. When a host is attacked by two or more of these mosaics, failure to distinguish them doubtless accounts for many of the discrepancies in the literature of the subject. This segregation of mosaics into distinct species is an important contribution to the investigation of this perplexing group.—J. M. C.

[10] TIFFANY, L. H., The filamentous algae of northwestern Iowa with special reference to the Oedogoniaceae. Trans. Amer. Micr. Soc. **45**:69–132. 1926.

[11] FERNOW, K. H., Interspecific transmission of mosaic diseases of plants. Memoir 96, Cornell Univ. Agric. Exper. Sta. December 1925.

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